The First Effective Method of Analysis for the Development of Ionochromic Azo Dyes for Aqueous Halide Detection

Honors Research Thesis

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by

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Abstract

The environmental, industrial, and biological presence of halides requires versatile and sensitive detection methods. Targeting the development of a colorimetric indicator for anion detection requires probing the response to proton concentration, activity, solvent, and other anions. While current research recognizes the variable effects, standard methods to quantize these variables do not exist. The standard methods developed were used in a survey of the colorimetric properties of commercially-available azo dyes conducted in the presence of halides to target structural features for a halide indicator independent of pH effects. Sudan Black B revealed unique spectral shifts with quantitative additions of bromide independent of solution pH. Coupled with molecular modeling studies, imperative structural and electronic features critical for anion sensitivity are under continual investigation. Specifically, analogs of Sudan Black B were synthesized in efforts to optimize solubility and structural simplicity, and these analogs exhibited bromide sensitivity in acid aqueous medium and have been further tested. Ultimately, with the development of a halide selective indicator for aqueous solutions halide concentrations could be measured with extreme accuracy and precision at a much lower cost. This method of detection could also be applied to similar anions that present analogous detection difficulties in aqueous solution. The flexibility of a molecular indicator allows for detection in a variety of environments including human cells, more specifically measuring halide gradients.

Chapter 1

Introduction

Anions play a critical role in the vast majority of physiological, environmental, and industrial processes integral to daily life. Due to their ubiquity, research in the field of anion detection has uncovered a multitude of phenomena with the capabilities of being able to detect and quantify the presence of these ions. Detection techniques span from the instrumental to the molecular scale with each one designed to push the constraints of environmental testing limitations. Success in this field has led to advancements in industrial quality control and development of physiological sensors.[1,2,3,4] However, advancement in supramolecular anion detection continues to be hampered by the expansiveness a robust technique would need to cover in order to be truly revolutionary thus leaving many opportunities for further innovation.

Halide detection was the main focus for a number of valid reasons. Specifically, halide detection parallels the same importance as broad anion detection when considering the role halides play in biological processes. Furthermore, halides provide a relatively easy model for anion detection as a whole due to the ease in working with their salts and acidic derivatives. Finally, the targeted anions bromide, chloride, and iodide each illustrate unique applications necessitating a versatile indicator.

Chloride has long been a well-recognized anion in seawater, biological cellular processes, and equally notable in an industrial capacity. Several industries, principally in the

chemical industry, utilize steel as an important construction material for the handling of numerous solutions. Specifically, hydrochloric acid exposure, even at a diluted acidic condition, can corrode steel over time. Industrial use of an indicator in these harsh conditions would help to quickly assess the presence of chloride and lead to a more efficient address of its effects. An easy to use molecular indicator may also alleviate the need for any extensive knowledge to achieve proper utility.[5]

Bromide provides a similar presence in several settings including in the fresh water-table and in soils. Methyl bromide, in particular, has a field use in soil fumigation relating to greenhouses and dry food goods. As a result, methyl bromide's usage leads to a vast exposure of the penetrating chemical to animals, plants, and ultimately the humans applying and handling the chemical. In consideration of exposure toxicity, several investigations have discovered the dangerous effects as a result of prolonged and unregulated exposure. Therefore, constant monitoring of bromide levels is necessary in this chemical environment along with the need to do this in variable circumstances (i.e. climate changes, aqueous medium) dictating that the indicator process overcomes such limitations.[6,7]

Iodide, though less prominent than its other halide family members, still provides reasoning for detection particularly in more complex environments. At a physiologically strict level, iodide is essential, yet at escalated levels it begins to become extremely toxic. Studies show that animals with elevated iodide presence in thyroid cells can lead to apoptosis through an oxidative stress mechanism. Asides from this biological presence, a more topical environmental impact is illustrated by the meltdown of the Japanese Fukushima nuclear reactor

in the wake of the tsunami in March of 2011. This demanded large scale detection in soil, water, and foodstuffs over a great distance for a sustained period during recovery. Therefore, This type of environmental need underlines the need for a flexible efficient aqueous detection method.[8]

With the vast set of conditions in which a halide selective detection technique is of great need, the field faces a monumental challenge of accommodating each of these variable environments. Currently, halide detection can be achieved with some success using various techniques including ion selective electrodes (ISE), ion chromatography (IC), and fluorescence indicators. Ion selective electrodes are considered a highly utilized potentiometric instrumental approach to anion detection in general.[9] However, despite their selectivity they are not always specific to one anion; more often they are *most* specific to a certain anion more than any other. An often cited example of this is seen with a fluoride ISE in which the membrane is most sensitive to fluoride, yet also responds to hydroxide at one tenth the response level of fluoride. Due to this issue and ultimately the ever-present matrix effects that accompany many complex solutions, ISE techniques become fairly complicated in accounting for the activity of a sample. Ion chromatography, a high performance liquid chromatography (HPLC) technique, provides improved sensitivity compared to ISE, but at the expense of an increased cost and labor intensive responsibility. Fluorescence halide detection has been established as a viable molecular technique via a quenching mechanism, however is subject to the same criticism that the previous two techniques face in that all three are limited by a need for instrumentation for analysis.[4] Furthermore, fluorescence lacks selectivity to some extent as the quenching mechanism of chloride is potentiated by the presence of bromide and iodide. With that in

mind, a detection method independent of instrumentation while providing ease and efficiency is deemed most advantageous.[3]

A separate approach to consider is the use of ionochromic indicators that are defined by the characteristic color change elicited by the presence of specific ions—this makes this detection technique possible without the need for instrumentation. Ultimately, colorimetric detection is the basis of naked eye detection allowing the eye to act as the detector. Furthermore, the response time and overall lifetime is directly competitive to the previously mentioned techniques. Thus far, a number of reports in this field have been published yet fewer examples exist where the sample environment becomes more competitive.[10,11,12,13,14] While the challenge of an aqueous testing environment is immense, the number of alternative interfering variables is also prohibitive and demand attention to conjecture the true precision and accuracy of a successful colorimetric indicator. To address the interferences, those variables need to be considered at every stage of research and development, particularly when considering the signaling mechanism and the structural components of the molecular indicator.

A typical colorimetric indicator is composed of two fundamental motifs that function to interact with the ion of interest and subsequently indicate this presence through visual color change. This is a general description and can imply varying structural complexities. A multitude of structural features have been proposed to interact exclusively with halides, but the structural feature that acts as the colorimetric indicator is typically a molecular dye.[1,15,2] The colorimetric properties of these dyes stem from an extensive system of conjugated pi electrons that delocalize across the molecule. If an anion perturbs ("disrupts") this electronic environment a

color change would be elicited. This particular means of detection allows for naked eye detection yielding qualitative and semi-quantitative determination of an ion's presence, limits the need for expensive instrumentation, and increases the flexibility in the environment. The technique described is similar to a pH indicator where a scale of color change is correlated directly to the proton concentration in solution. Understanding that, the spectra indicates changes in the electronic environment (i.e. color change), ultraviolet-visible (UV-Vis) spectroscopy is utilized to quantify concentration with increased precision, and the linear instrument response can be used for concentration determination through established calibration response.

When considering the structural components used for colorimetric anion detection a number of organic functional motifs have been implicated in literature. A myriad of solution introduction methods also exist so various structural components have been designed with the intention of acting similarly to a membrane or with the intention of environmental introduction via a polymer/plastic medium.[16,17,18,4] The well represented fluoride sensors in particular have a sensing motif consisting of an anion binding receptor that typically incorporates pyrrole, amine, and phenol functional groups and a component that changes color in response to the binding event. The mechanism of fluoride detection consists of a noncovalent hydrogen bonding like interaction and when present in excess may cause a deprotonation-like event.[12,14,19,20,21,22,23,24] Because of the nature of this interaction, the anion is extremely difficult to detect in competitive media and is treated somewhat separately from the remaining halides. However, the structural motifs from this literature are still utilized across the field and are worth investigating.

Beginning this investigation, an expansive literature review of the key structural motifs that have achieved success via a variety of parameters including colorimetric detection, selectivity for anions, and aqueous or competitive media capable was conducted. The results of this study yielded a number of synthetic avenues for exploration, some of which are further detailed. A discussion of these structural motifs would be incomplete without representation from models such as porphyrins, calixarenes, bis functionalized molecules and naphthalimides.[25,26,27,11,1,28] By exploring these specific examples, a number of important interactions varying from covalent bonding to non-convalent interactions such as hydrogen bonding are seen.[29,30]

The basket-like topology of calixarenes present a more structurally-complex anion sensing structure. These molecules' derivatives include four and six substituted structures that are typically tailored to interact with a species, and present a highly-ordered structural "cavity". A particular study of anion recognition through a preorganized tetra-amidurea derived calix[4]arene molecule (Figure 1) illustrates the unique anion interactions with these structures along with the structural flexibility this particular motif possesses. These calixarenes molecules typically offer interaction sites either within the calix cavity or through a non-covalent avenue with the substituents. The particular example pictured below offers an "on-off" type colorimetric indicator in polar solvents particularly for fluoride and pyrophosphate anions. The urea moiety is equally valuable, as this is a popular anion binding structural component seen throughout the literature. By incorporating both of these features, the opportunity for anion interaction improves dramatically.[27]

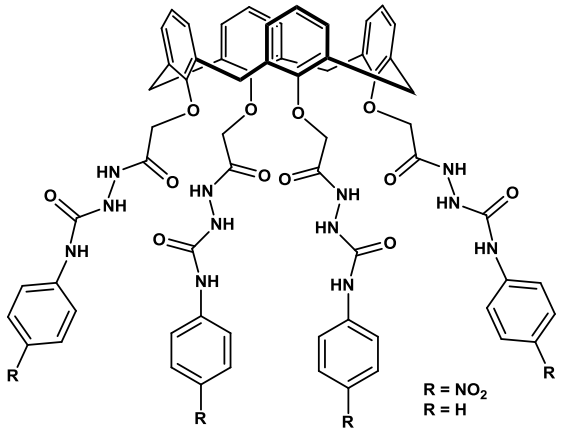


Figure 1. Tetra-amidurea Derived Calix[4]arene

A similar study employs the calix structure as a calixpyrrole anion receptor (Figure 2) for competitive media chloride detection. The hydrogen bonding of the amine group in this molecule was hypothesized to be advantageous for anion detection and was demonstrated to bind fluoride and chloride in a DMSO-H_2O binary solution. Furthermore, by finding detection utility with the pyrrole functional group, this study further expanded the range of possible successful anion indicator moieties.[11,31,32,33,34,35,36,37]

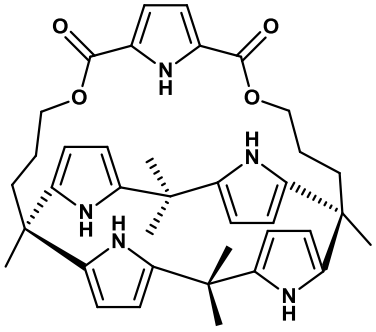


Figure 2. Calixpyrrole

Like calixarenes, naphthalimides provided a basic structural element from which chemosensors were developed. The applications and modifications of this functional piece highlight a variety of invaluable components to the field of supramolecular sensing. Specifically 1,8-naphthalimide (Figure 3), is a functional basis for a vast number of synthetic variations on chemosensors that has been an integral part of the literature. A review on the use of this structural building block was completed citing fluorescence and colorimetric uses rooted in diverse functionalization. The basis for the use of 1,8-naphthalimide is its characteristic photophysical properties of absorbing its long wavelength (UV-Vis capable) emission and its ease in synthetic substitution.[1,2,20,38]

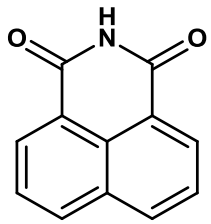


Figure 3. 1,8-naphthalimide

In their review, Gunlaugsson *et al.* discusses typical construction schemes for sensors citing a binding unit-signaling unit or binding unit-spacer-signaling unit makeup. The signaling unit is often a colorimetric or fluorescent molecule defined by highly conjugated aromatic compounds such as porphyrins or azobenzenes (dye). While it seems organized to categorize such organic structures as one or the other, further discussion of particular moieties such as porphyrins will lead to a blurring of the line between binding and signaling unit. Asides from

that particular categorization critique, the review functions to highlight a string of implicated structural components integral to sensing in conjunction with groups such as naphthalimide.[1]

Of the multiple applications of the naphthalimide structure, the bis functionalized sensors are underlined and described as two fluorophores or chromophores attached to a covalent spacer. The naphthalimide variety of these structures is illustrated by utilizing a familiar structural component in thiourea from the 4-amino varieties of the naphthalimide structure. The disubstituted molecule below (Figure 4) operates as a fluorescent anion sensor with notable absorption changes in the presence of multiple divalent anions. The sensor's ability to offer absorption and emission information provides strong synthetic support for utilizing fluorophore based molecular structures—the dual chromophore properties are valuable.[1]

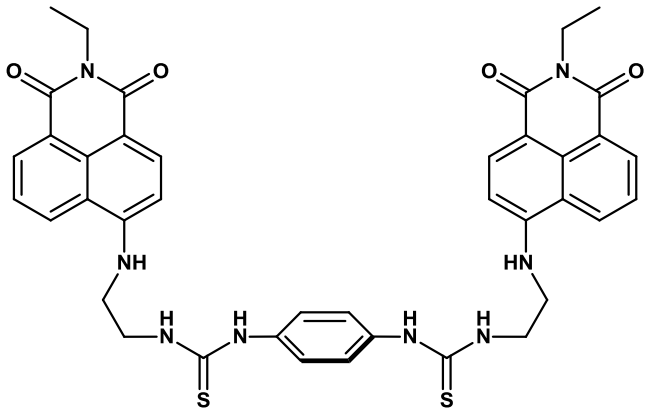


Figure 4. 4-amino Bis Functionalized Naphthalimide Structure

A discussion on the particular advantages of using a bis-functionalized system, and how the conformational properties can yield positive binding events is highlighted with amide and

urea functionalized dithienylethene (Figure 5) utilizing photochromism. Photochromism is illustrated by the process of irradiating a species with UV-Vis light leading to a structural configuration change which is the basis of a switching system. Due to this structural change, it is reasonable to assume that the sensor might have variable anion affinities. The particular molecule shown in Figure 5 demonstrates this by having switchable affinities for chloride and bromide depending whether the open or closed ring isomer structures exist in solution. The structural example is extremely pertinent to highlight avenues of achieving multi-anion detection with a single sensor while preserving selectivity to some extent. This molecule also demonstrated the ability to easily reverse between two states with consistent response over ten repetitions and is a unique application for both the functional group dithienylethene and more broadly for bis based molecules which allowed for varied molecular configurations and selectivity.[28]

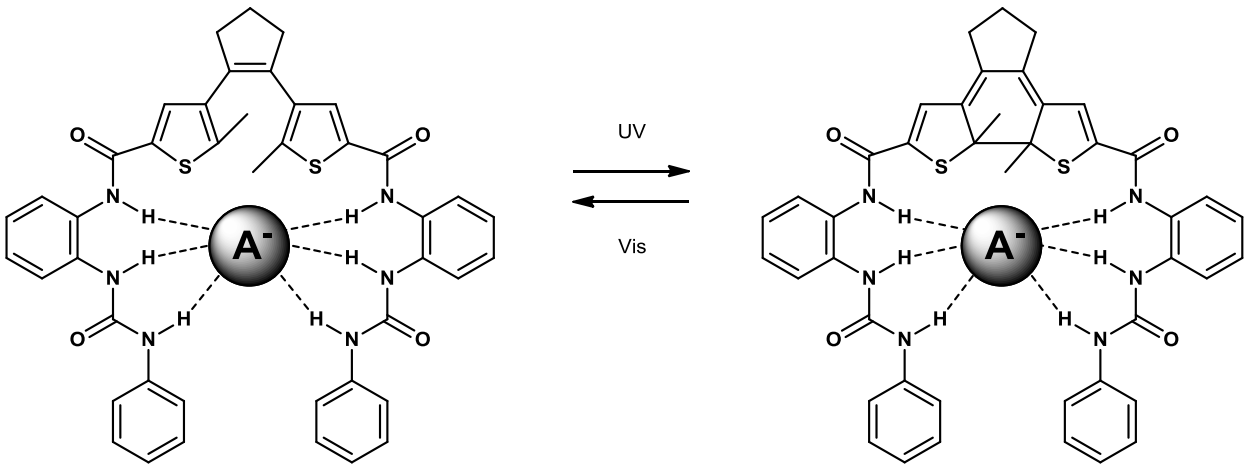


Figure 5. Amide and Urea Functionalized Dithienylethene

Porphyrin structures as mentioned, in concurrence with the naphthalimide functional group, provide a unique molecular environment characterized by their macromolecular, cyclic, and highly conjugated structural features. Worth noting is that these particular properties provide an advantageous detection avenue due to strong visible absorption bands; studies suggest this organic structure may operate as more than a signaling unit. Porphyrin halide detection is a variation on molecular size-based detection that operates largely through a quenching mechanism. Despite the change in response (fluorescence versus visible), similar anion binding units utilize the hydrogen bonding interaction. This is illustrated with the tetrapyrrole group in the porphyrin structure that, in a protonated variety, undergoes a noncovalent interaction with anions and leads to a rather intense fluorescent response. Extensive work has been completed forming the porphyrin below (Figure 6) in an effort to detect iodide ions through a fluorescent quenching mechanism. The data supports a trend with change in fluorescent wavelength (color) as a function of iodide concentration.[25]

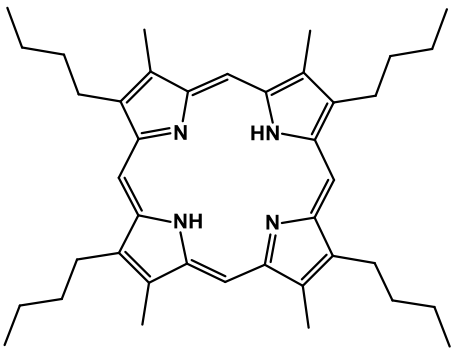


Figure 6. 3, 7, 13, 17-tetramethyl-2, 8, 12, 18-tetrabutylporphyrin

The complexity of molecular anion detection, even when narrowed to halides facilitates the extensive variation of functional moieties highlighted in the literature. Even those more

explicitly discussed, illustrates the vast synthetic approaches that have had some success. However, to facilitate further improvement, the question becomes at what point are these molecular indicators limited? Since, the field of aqueous detection is still relatively uncharted, it is not unreasonable to assume that much of the research has clear limitations both inherent and/or entirely overlooked due to difference between experimental and goal environments of detection. The current literature is in large part still attempting to make the transition from organic to aqueous detection, therefore a complete and consistent success metric must be constructed. The development of a colorimetric indicator for anion detection must include consideration of the response due to proton concentration, activity, solvent, and competing anions. While current research recognizes the variable effects, standard methods to quantify these variables do not exist.

Within the structural examples explored, many variables are indirectly addressed or ignored. Specifically, the popular polar organic solvent DMSO has a high dielectric constant comparable to water making it a reasonable introductory detection testing medium. However, the assumption that a smooth transition exists between DMSO and water may be presumptuous and is demonstrated by those utilizing binary solution mixtures.[13,11,28,12,14] Fluoride detection in particular faces difficulties as the aqueous environment is introduced, and water outcompetes the anion in the desired interaction—this is a thoroughly addressed limitation in the literature.[2,12,14,39,40,41] The extent of this issue indicates the great challenge in crossing over into a novel competitive media such as water.

A similar point is made with the consideration of the effects of proton concentration. An interesting observation in reviewing literature is that many of these colorimetric indicators bear resemblance to other dyes with various other functions. Methyl red, a known pH indicator, is remarkably similar to methyl orange—it would therefore be expected if this molecule also demonstrated pH effects. While the shared structural features may end at similar chromophore properties, it is not practical to disregard the possibility that methyl orange may also be affected by a change in proton concentration.[10,13]

Aqueous detection promotes variables such as proton concentration, pH, activity and solvent effects to equal importance with selectivity and sensitivity of anions. It is impossible to ignore the limitations and possible interferences that these variables may impose on a desired effect. The goal of such critique is not to project unrealistic expectations of what success might mean in designing and implementing a colorimetric anion sensor, rather it is to explicitly report the true success of each sensor and the limitations that inevitably exist. Standardizing this practice would only improve the structural repertoire of detection avenues as it would be better understood which molecules or functional moieties contribute or hinder a desired interaction. Furthermore, by employing rigorous testing methods, the ability to better understand mechanisms of detection is greatly enhanced.

Through extensive literature review and analytical critique, the success metric was determined for the design of an ionochromic aqueous halide indicator. While several promising indicators have been developed, the need for continual improvement is clearly evident by the diversity of anions and testing environments coupled with the multitude of competing

variables. Developing molecular halide indicators provide both a useful collection of colorimetric indicators as well as insight into a broader subject. Through addressing of both structural components of the indicator along with the environmental limitations, success in colorimetric halide detection can be better characterized and further utilized to develop a comprehensive model for general anion indication.

Chapter 2

Analytical Methodology Introduction for Aqueous Colorimetric Indicator Development:

There are a number of variables in aqueous solution that could affect the response of a colorimetric aqueous halide indicator such as pH, solvent effects, competing ions, activity changes, and solubility. These variables tend to be interconnected and have been addressed intermittently in the literature with no standardized metric for success. More often than not, concrete statements about the success of these indicators are very limited or often overstated.

While setting limitations may seem to diminish the success of an indicator, it actually provides a more accurate description of the structure's capabilities and its applicable detection environment. This information is imperative for the accurate application of these indicators as well as for structural improvements. Application limitations are vital when specialized dyes have a targeted utility, but might face response issues if not properly characterized, particularly when the desired response is in a narrow dynamic range or affected by high or low pH.[42, 8,43,1, 7] Specific adjustments could be made to properly use these indicators provided that the limitations are explored and clearly stated. Furthermore, the extent of the environmental changes may later affect solubility and response, therefore it needs to be directly addressed as these changes may lead to drastic response interferences.

A literature survey and critique pertaining to the way these variables are addressed for methodology development and improvement was imperative. While the shortcomings in characterization were apparent in many cases, it most likely occurred due to the nature of the

testing environment. Colorimetric aqueous halide detection presents a number of novel challenges and the mechanism of action is not always well understood. The complex issues are exacerbated by the challenges of a highly competitive media that, at the very least, hinders indicator solubility. As such, it is expected that a metric of success has not yet been fully realized; there are multiple challenges to overcome in an aqueous medium. The following survey of the variables and the subsequent literature support of their importance is not exhaustive and prioritizes the most difficult to overcome.

Proton concentration is a fundamental consideration for a protic detection environment and as such may affect detection methods sensitive to these interferences. This measure is reported as a pH effect and is rarely tested directly outside of fluoride and molecules detected via a similar mechanism (cyanide).[40,39,12,14,44] This is particularly interesting given the model that many employ for the colorimetric or fluorescent signaling unit. Many of these models incorporate structural components containing amines when protonated or deprotonated lead to vastly different behavior.[23,22] Therefore, it is likely that a color change correlated to pH changes may interfere with any anion interaction signal. Most signaling units have analogous structural components to common pH indicators particularly when utilizing an azo functionalized dye as part of the indicator motif. An example of this usage is a molecule like methyl orange which has functional groups related to methyl red (pH indicator) and therefore is undoubtedly affected by large pH changes.[10]

An alternative to considering pH effects on indicator response in the literature are to bypass this issue altogether. Many indicators are tested in buffered solutions largely

eliminateing the pH effects, but by extension these indicators are limited to these buffered environmental conditions. With that being said, they are still able to make successful indicators, yet the limitations are clearly present and need to be stated as such (many physiologically based indicators test in buffers—understandably).[18,42,13]

Solvent effects are an aspect of indicator testing that are usually considered, however variably acknowledged. Most often this comes about by first testing in a less competitive environment such as a relatively nonpolar organic medium and then subsequently testing the same indicator in a more competitive medium like DMSO.[11,13,14,20,21,27,45] The logic behind this procedure is that the indicator's response would transcend environmental barriers. However, a caveat must be placed on the assumption particularly due to the structural nature of these molecules. These organic dye molecules typically constitute at best variable solubility in aqueous environments. By stating that an indicator would be successful in aqueous medium due to successful results in binary DMSO-water solutions is overstated. It is not unusual to have a varied response across solutions; this phenomenon often times is not addressed in the literature. Furthermore, if solubility limitations dictate the need for structural changes, they would also affect the indicator response. Therefore, if the indicator is meant for an aqueous application, the most reasonable testing environment is an aqueous medium—if this is not an immediate possibility but probable in the future, the limitation must be stated as such.

Relating the effects of proton concentration and solvent is solubility issues. How solubility is addressed depends on the intended function of the indicator as it relates to the targeted application environment. The vast pH scale also leads to a large variation in how the

molecule may interact in solution.[46, 47] Therefore, it is likely that altering proton concentration will affect the solubility of the indicator molecule—particularly that which is prone to protonation and deprotonation effects. Further examples, include changing from an aprotic organic solvent to an aqueous medium; this not only changes the environment but also affects the solubility of an organic dye. The future work of a multitude of studies includes improving solubility through synthetic modifications, which would also undoubtedly affect the indicator response and therefore should be taken into account.

Competition of other anions is a fundamental consideration the literature consistently probes as it is both rare and thoughtless to propose a colorimetric that is not tested with at least the most similar competing anions. The standard method in the literature is to test the salt form of the anions with the counterion as an organic species such as tetrabutyl ammonium.[14,48,49,21,50] While this is perfectly acceptable and does provide a clear picture of what might interfere with the response with respect to the anions, improving this testing procedure would involve including cation changes as well. While the indicators are not expected to respond to cation change (or cations at all), it is not an unreasonable interference to anticipate, as it is analogous to proton concentration effects.

Finally, a variable circumvented almost entirely in the literature is activity changes. Activity is a realistic measure of the concentration of the species in solution particularly in relation to the other species (accounting for equilibrium). This is often expressed as a measure of ionic strength, which varies depending upon the ions in solution. This particular variable is to some extent addressed by considering changes in the introduced ion concentration or identity,

yet fundamentally it is a variable that should be directly considered. Activity changes may be at the core of an indicator's response and could undoubtedly create an unexpected or inaccurate concentration response. A clear difference between the activities of a solution might be illustrated by the difference in ionic strength of blood or tissue versus drinking water. While drinking water is not completely free from all competing species, blood contains a number of salts, ions, and is buffered. The activity characteristics of these environments are drastically different and reasonably an indicator response would be different as well. An aqueous testing environment is a broad and challenging region offering a number of inherent variables that may interfere with a colorimetric indicator's ability for accurate detection and as such should be thoroughly characterized.

Due to the importance of proton concentration, solubility, activity, and competition of other anions, it was necessary to consider an assay that would qualitatively and quantitatively characterize the anions' effects for the purpose of not only limiting the field of prospective indicator models, but also for accurately portraying the quality of perspective novel indicators. The particular testing method herein was designed to target all of the highlighted variables and was applied to a qualitative analytical survey of commercially available azo dyes for exploration of a colorimetric halide effect primarily independent of pH. This survey focuses on the azo functional family due to the prevalence of these molecules as dyes. The purpose of the study was to not only identify an effect, but also to better understand structural features that might be applied to the development of novel colorimetric halide indicators for aqueous detection. The more rigorous methodology supports this goal through the characterization of the

commercial dyes, which could lead to a mechanistic understanding of colorimetric anion detection.

Commercial Dye Survey Experimental Procedure:

UV-Vis Spectroscopy Instrumentation:

UV-Vis spectral analysis was completed with a Varian Cary 5000 UV-Vis-NIR spectrophotometer scanning 175 nm-800 nm at 600.00 nm/min.

UV-Vis Qualitative Commercial Dye Survey procedure:

Solutions were prepared with a proposed solvent by dissolving 10-15 mg/L of the targeted dye adjusted for 0.5-1.0 absorbance. Additions of acidic halide were added incrementally with a spectrum recorded after each addition until the solution was extremely acidic. The pH and activity were tested with subsequent additions of NaOH and a spectrum was recorded after each addition until the solution was extremely basic. Each acidic halide and a salt form of the halide (KBr, NaCl etc.) was tested and compared for spectral differences. If water was not the testing medium, several organic solvents were utilized for comparison and to better understand the solution behavior.

Legend Key:

Sudan Black B: SBB, Janus Green B: JGB, Sodium Hydroxide: OH, Methanesulfonic Acid: MSA The incremental numbers indicate the order of addition and typically honor a 10-20 µL addition into a cuvette of dye solution.

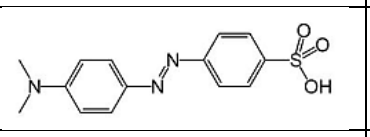
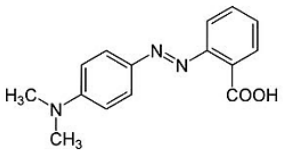
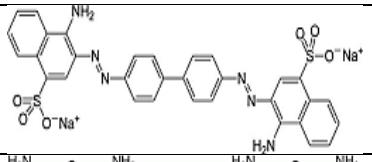
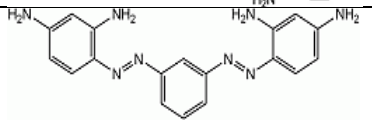
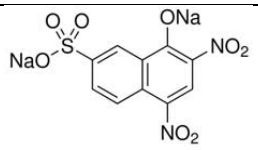
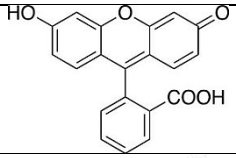
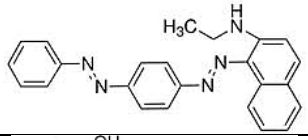
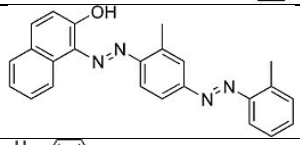
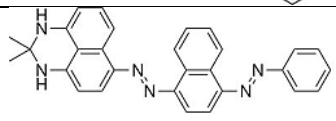
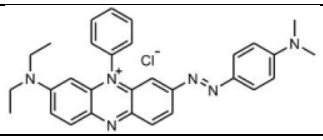
Analytical Commercial Dye Survey Results:

The analytical methodology developed for the testing of aqueous colorimetric halide indicators was realized in an effort to establish a subset of commercial dyes with some identifiable effect in the presence of halides independent of pH. This commercial dye survey served the function of narrowing the field of functional moieties with successful halide responses while better understanding how certain variables like proton concentration might affect detection response. The study was centralized around azo-functionalized dyes with some notable exceptions for structural variety and comparison.

When evaluating a possible commercial dye candidate, competing variables were prioritized according to specific goals. The most fundamental demand of a successful dye was identifying a spectroscopic change in wavelength the presence of a halide. This was then directly compared to any spectroscopic response from the addition of acid and base as many of these molecules resemble notable pH indicators and therefore might produce a competing or convoluted response with pH changes. Finally, variables such as solvent changes, solubility, competing anions, selectivity, and sensitivity were explored and evaluated for further structural utility. The qualitative nature of these tests allowed for quick and efficient data acquisition that supported the pursuit of several molecular motifs. The breadth of data collected combined with literature observation supported the presence of interference from the pertinent variables considered and the subsequent need for a standardized colorimetric aqueous indicator testing procedure.

The following table details that the most germane data collected from a representative set of ten dyes qualitative and/or quantitatively tested. While the majority of the commercial dyes are azo-functionalized, fluorescein and naphthol yellow are two dyes representing the structural motifs more commonplace in fluorescence. Thesewere explored in an effort to consider the literature supported dual properties of fluorophores as chromophores.[1,2] Initial observation of the results immediately indicates the profound influence of proton concentration on many of these dyes. This is relatively foreseeable but does highlight the need for pH and its effects to be identified as a possible legitimate limitation for most dyes.

Table 1. Commercial Dye Survey Results

Commercial Dye	Chemical Structure	Solvent	Tested Ions	Major Effect
Methyl Orange		THF, CH_3CN, H_2O	H^+, Cl^-, I^-, OH^-, Na^+	pH
Methyl Red		THF, CH_2Cl_2, CH_3CN, MeOH, H_2O	H^+, Cl^-, I^-, K^+, OH^-, Na^+, Br^-	pH
Congo Red		MeOH	H^+, Cl^-, Br^-, I^-, OH^-, Na^+	pH
Bismarck Brown		H_2O, MeOH	H^+, Cl^-, I^-, OH^-, Na^+, Br^-	pH (basic) possible I^-
Naphthol Yellow*		MeOH	H^+, Cl^-, I^-, K^+, OH^-, Na^+, Br^-	pH
Fluorescein*		MeOH	H^+, Cl^-, I^-, K^+, OH^-, Na^+, Br^-	pH (basic) possible I^-
Sudan Red 7B		MeOH	H^+, Cl^-, I^-, K^+, OH^-, Na^+, Br^-	NA
Sudan IV		CH_2Cl_2	H^+, Cl^-, Br^-, I^-, OH^-, Na^+	NA
Sudan Black B		CH_3Cl_2, CH_3CN, DMSO, MeOH	H^+, Cl^-, I^-, K^+, OH^-, Na^+, Br^-, TBA^+	Halide, Br^-
Janus Green B		CH_3Cl_2, THF, CH_3CN, H_2O	H^+, Cl^-, I^-, K^+, OH^-, Na^+	Halide, Cl^- pH (small, basic)

The following detailed analysis focuses primarily on those dyes that were deemed successful, Sudan Black B and Janus Green B. They were most extensively tested, emphasize the importance of the analytical methodology, and were targeted in novel dye development.

Sudan Black B Qualitative Studies and Discussion:

When approaching a molecule like Sudan Black B, the first consideration in testing its applicability for aqueous halide detection was dissolving it in a solvent which was as similar to water as possible. In this particular case, the dye was insoluble in water and therefore was tested in CH_2Cl_2 and DMSO for solubility ease and comparison. DMSO specifically provides a better characterization as it hs a comparable dielectric constant to water with the benefit of still being an organic medium. Once dissolved, the dye solution was tested for spectroscopic changes in the presence of incremental additions of acid, base, and halides. Figure 7 details the results from the dye-halide tests in CH_2Cl_2 which fueled the need for continued study of Sudan Black B.

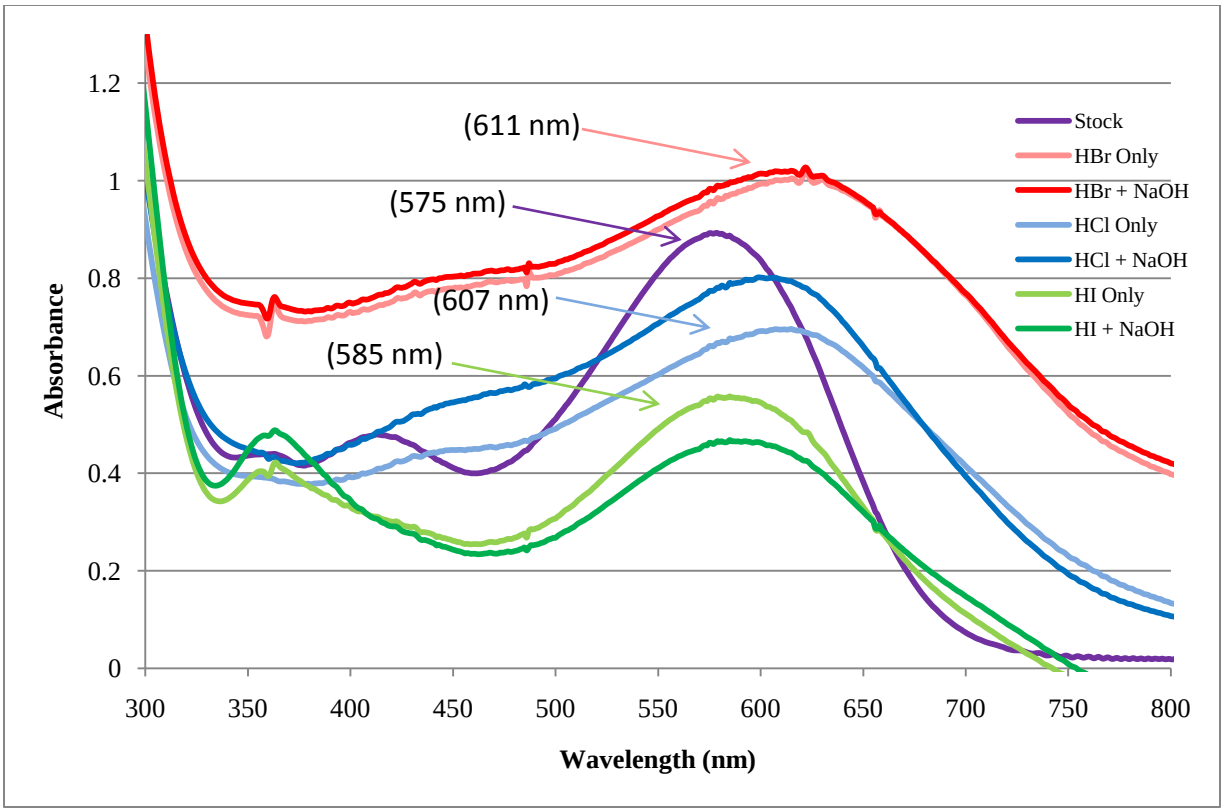


Figure 7. Sudan Black B in DCM spectral response to additions of bromide, chloride, iodide, and base

The initial data does not immediately support a selective halide effect, but what is worth considering is the distinct but unequal shift in the presence of each halide. The additions of bromide and chloride both elicit a substantial response and the presence of iodide still elicits a perceptible shift. While this result might be dismissed as non-selective and extremely limited by the vastly different environmental conditions, the comparison to the well-established halide fluorescent detection mechanism provides support for continued study. This mechanism often shows detection of chloride through quenching, bromide through a more intense quenching, and iodide through an even more extreme quenching responseA trend in the spectral shift of

the λ_{max} is also apparent with added halides therefore worth further pursuing in a more analogous solvent system to an aqueous medium.

While a halide effect was the primary targeted result, the effect of proton concentration was also considered by comparing the additions of acid, acid halide, and tetrbutylammonium (TBA) halide and it became easier to distinguish the contributions to the colorimetric effect observed. The results provided both an enlightening and challenging view of Sudan Black B in CH_2Cl_2 (Figure 8). The spectral data provided support for a halide induced effect via a similar red shift of the peak maximum in the presence of bromide that was added as the acid bromide and TBA bromide . However, Sudan Black B in the presence of methanesuflonic acid also resulted in a 49 nm red shift from the original peak maximum. While this is recognized as a competing effect, the tetrabutylammonium bromide salt addition had an effect independent proton concentration. Though not easily separable the dye was considered for further investigation with the understanding that acid also participates in the resulting effect

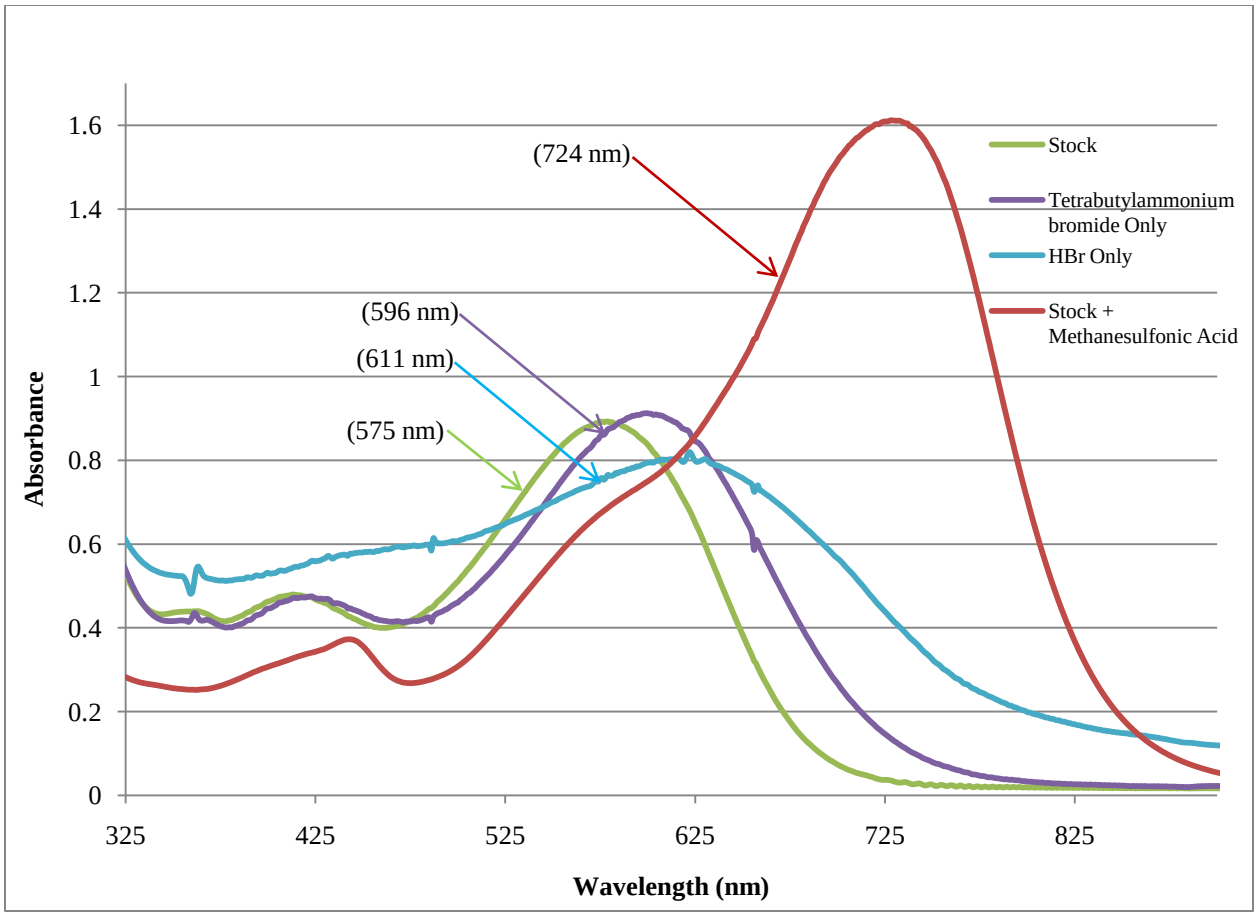


Figure 8. Sudan Black B in DCM spectral response to additions of bromide, acid, and TBA bromide salts

Addressing the original methodology concerns, several variables were considered with varying results. Sudan Black B provided a colorimetric response in the presence of halides, which was the primary goal of the study. However, it was also affected by the presence of acid and was insoluble in water. While this was disconcerting to some extent, positive results with the addition of halide salt and the inconsequential response to basic additions provided support for continued study. Furthermore, the CH_2Cl_2 solvent system was not a similar enough system

to water to inspire immediate structural implications and therefore instigated further testing in a more enlightening medium.

To address solvent concerns and further probe Sudan Black B's halide response, a more competitive solvent system was explored through the use of DMSO. While DMSO is still an organic medium, it does provide a comparable dielectric constant to water and was used in literature as a comparable detection environment. The resulting studies in DMSO proved equally interesting with notable differences from the previous CH_2Cl_2 data (Figure 9).

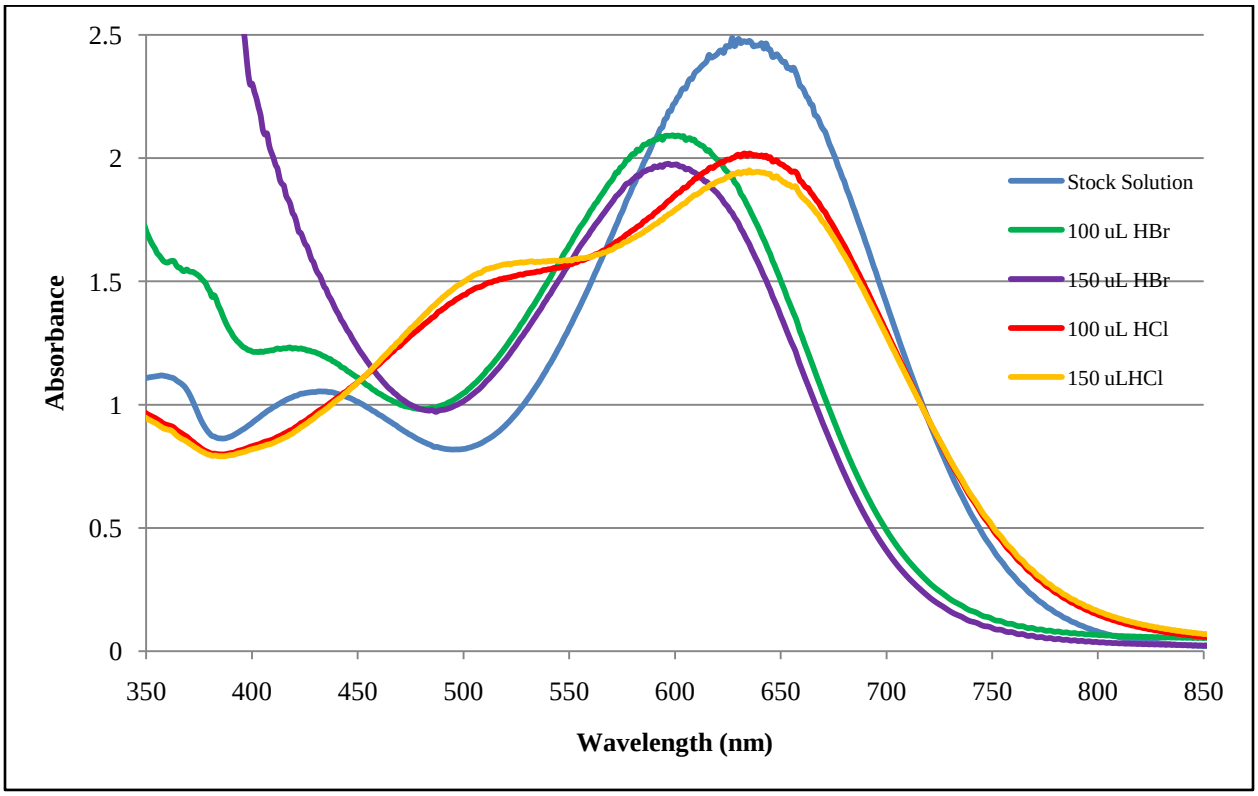


Figure 9. Sudan Black B in DMSO spectral response to additions of bromide and chloride

The most notable contrasting result was the direction in which the peak maximum shifted upon the addition of bromide. The blue shift in DMSOwas originally a red shift in CH_2Cl_2 .This distinct

blue shift in the presence of bromide relative to the stock Sudan Black B solution is fundamentally a destabilizing effect relative to the stabilizing or lower energy shift of the halide additions in CH_2Cl_2. Furthermore, the once difficult to discern chloride and bromide effect was now differentiated to some extent. The acid chloride additions in particular resulted in very little shift in the peak maxima amount relative to the stock Sudan Black B peak. The shift in the presence of chloride instead resulted in a growing shoulder around 500 nm. This was an entirely different effect than what was seen with the acid bromide additions yielding a blue shift of the peak maximum of 36 nm relative to the Sudan Black B stock solution.

The following figure addresses pH effects of Sudan Black B in DMSO (Figure 10). The data illustrates a less convoluted effect than originally seen in CH_2Cl_2.

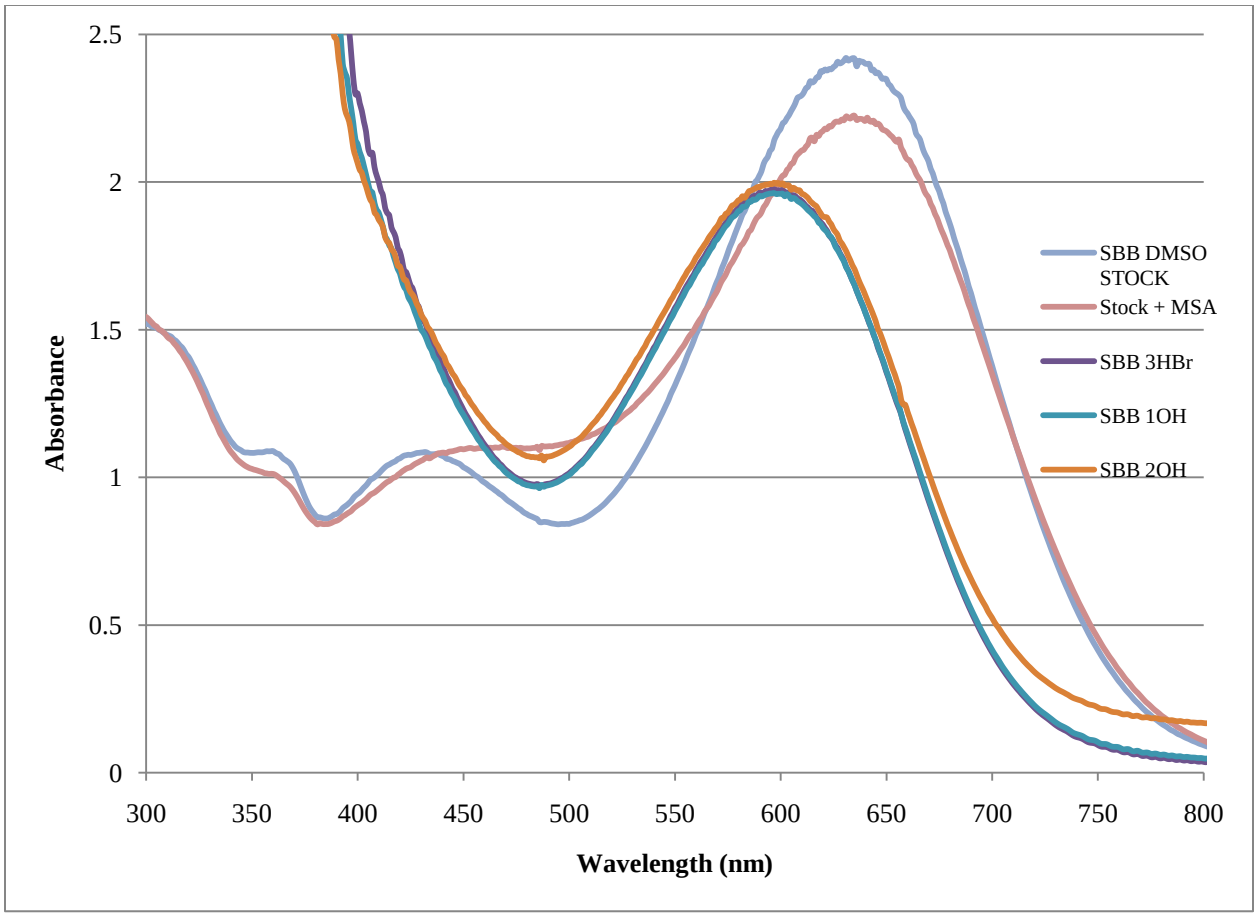


Figure 10. Sudan Black B in DMSO spectral response to additions of bromide, acid, and base

Additions of hydrobromic acid differ from the methanesuflonic acid additions as the methanesulfonic acid additions elicited little peak maximum changes. However, acid additions did spectrally affect the original stock spectrum with a growing peak at 485 nm.. This additional peak did not seem to interfere with the primary halide effect. Also, the spectral shift resulting from the bromide additions showed little change with subsequent base additions supporting the absence of a basic pH and activity effects.

The qualitative Sudan Black B-DMSO spectroscopic data revealed the importance of a number of the competing variables explored. Specifically, the comparison across the two

solvent systems revealed how the environmental makeup can drastically alter the response of such an indicator. The structural differences and interactions likely to occur between the solvent and dye support this conclusion, especially when considering the vastly different dipoles and non-covalent interactions of the two solvents employed. As such, halide introduction clearly has affected the dye-solvent interaction in a way that stabilizes or destabilizes the electronic transition observed spectrally. Competing anions also served as an accurate response deterrent and have been distinguished in the dye-DMSO tests through the comparison of chloride and bromide. Activity changes to the solution resulted in no noticeable spectral changes as several ions that altered the ionic strength were introduced with only the bromide and chloride additions eliciting any substantial spectral change. Finally, proton concentration was a contributing factor in the response seen in CH_2Cl_2, but the use of DMSO elicited little spectral change. However, this effect is not fully resolved as halide salt tests were inconclusive due to their low solubility.

The Sudan Black B qualitative halide tests were considered a successful commercial dye study. While little can be concretely concluded, the initial data does support a further probe through quantitative limit of detection studies. Because the studies will continue to be in DMSO, structural derivatives could understandably respond significantly different to bromide in aqueous solution. Nonetheless, characterizing this effect aids to better understanding the functional components of a response and would also allow the constructing ion ofa response curve relating peak maximum shift to halide concentration.

Janus Green B Qualitative Studies and Discussion:

A second commercial dye studied for a possible colorimetric effect in the presence of halides was Janus Green B. A number of differences are immediately obvious between the previously discussed Sudan Black B and Janus Green B, most notably the functional components of the structure. As such, it was expected that these dyes would vary in solubility and response. Janus Green B, unlike Sudan Black B, did not require a number of organic medium halide tests for comparison and support, as it was soluble in water. The qualitative halide tests (Figure 11) were of the first few that were completed for this project. As such, the analysis methodology has undergone a substantial evolution in testing procedure since then. Therefore, the data in figure 11 represents an early analysis of Janus Green B with acidic additions of chloride and iodide.

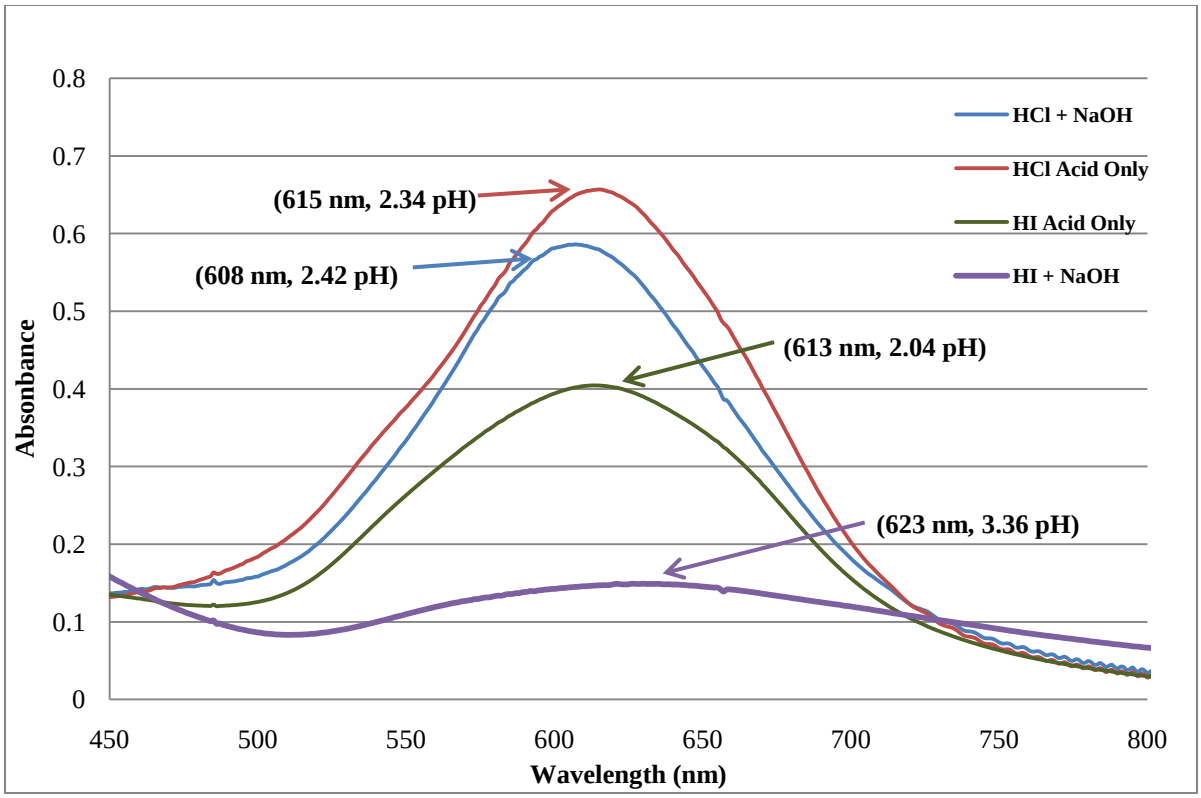


Figure 11. Janus Green B in water spectral response in the presence of chloride, iodide, and base

The data, while representative of the colorimetric effect desired and illustrative of pH as a possible interference, falls short of fully characterizing the possible variables affecting this it. The chloride and iodide additions show almost indistinguishable differences, but the iodide spectra absorbance and wavelength are severely affected by additions of sodium hydroxide (10 nm red shift). Theis change may be due to an activity effect as the peak maximum looks to be red shifting as the pH approaches 2.04, the original pH of the iodide exposed solution. The ideal testing comparison would have been to have an equal pH between the acid halide addition spectrum and additions of hydroxide-iodide spectrum as this holds the pH and halide concentration constant while allowing for a response to the change in ionic strength and

therefore activity to be observed. The proposed conclusions made are by no means more than hypotheses, to further data, and analysis and to improve methodology.

Janus Green B was again tested in water to further probe the chloride response which wasdeemed the less convoluted halide effect of the two tested and to further explore the implications of pH and chloride additions (Figure 12).

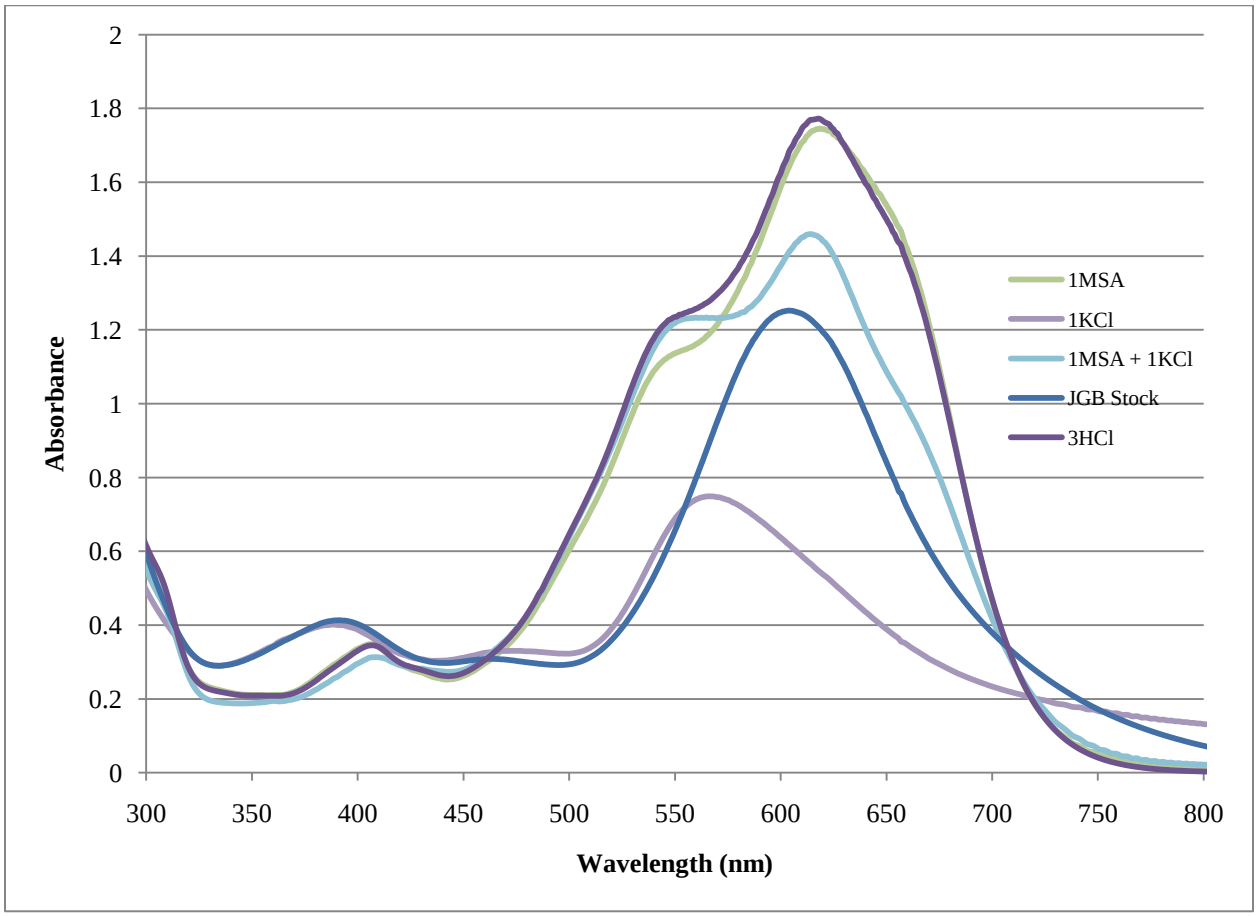


Figure 12. Janus Green B in water spectral response to the additions of acid and chloride

The spectra illustrated a somewhat complex effect in the presence of acid. The Janus Green B stock peak as compared to the potassium chloride addition spectrum shows a substantial blue

shift of about 40 nm. However, when compared to the acid chloride additions, the response becomes more complex with a larger peak with a newly formed shoulder at 543 nm. This shoulder was also present slightly blue shifted with additions of methanesulfonic acid (no halide) supporting that this particular spectral response is acid caused by to some extent. A further comparison of acid and potassium chloride additions together show a very similar spectral response particularly in the placement of the shoulder. Together this data portrayed simultaneous effects much like what was seen with Sudan Black B in CH_2Cl_2. There was clearly some contributing effect correlated to the presence of chloride, but the effect of acid presence could not be ignored or easily distinguished. Further analysis to probe the colorimetric effect in the presence of chloride as it relates to acid additions was completed (Figure 13).

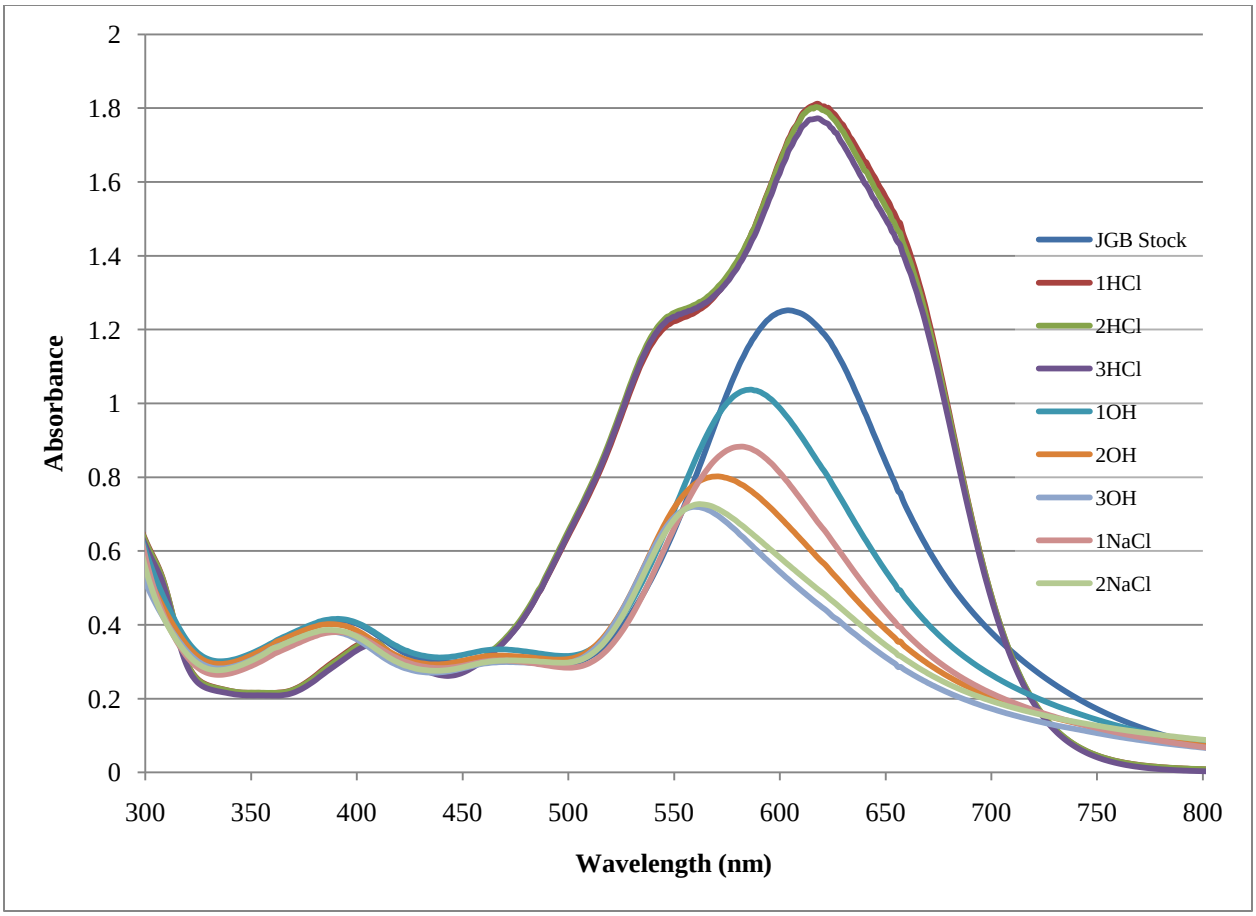


Figure 13. Janus Green B in water spectral response in the presence of acid, chloride, and base

This data presented a more dynamic view of the halide effect as it relates to pH changes. The first comparison was one already examined between the stock Janus Green B spectrum and hydrochloric acid additions spectra. These differ in absorbance and most notably in the shoulder that appears at 543 nm. This was clearly different from the sodium chloride addition spectrum that shows a distinct peak maximum blue shift relative to the stock Janus Green B spectrum. However,additional data was included in this test after the hydrochloric acid and incremental base additions to the solution were completed. These sodium hydroxide additions to the already acidic chloride-dye solution resulted in peaks that most closely

resemble the chloride salt additions. As such, it seemed as if the acid effect was reversed to reveal only the effect correlated with the presence of chloride. This was not conclusive due to the qualitative methodology, but did provide support for further characterization of Janus Green B's structural components contributing to the response of chloride in an effort to exploit them for further novel development.

Quantitative Limit of Detection Experimental Procedure:

UV-Vis Spectroscopy Instrumentation:

UV-Vis spectral was analysis completed with a Varian Cary 5000 UV-Vis-NIR spectrophotometer scanning 175 nm-800 nm at 600.00 nm/min.

UV-Vis Quantitative Commercial Dye Procedure:

The dye was dissolved in solution at 15 mg/L and subsequent halide-dye solutions were prepared volumetrically at multiple incremental halide concentrations. Sudan Black B solutions were prepared with hydrobromic acid in DMSO while Janus Green B solutions were prepared with potassium chloride in water. A spectrum was recorded of each solution and plotted as a response curve.

Quantitative Limit of Detection Studies and Discussion:

Both Sudan Black B and Janus Green B provided possible colorimetric halide effects worth investigating for synthetic novel indicator development. These successful results were not without ambiguity particularly in establishing the effect of proton concentration. However,

further characterization was deemed useful with the understanding of such limitations. The final analytical testing measures before synthetic development were to quantitatively describe the halide effect seen in previous qualitative tests. This spectroscopic analysis aimed to unmask a limit of detection, to develop mechanism hypotheses, and to determine advantageous synthetic models.

Quantitative description of the colorimetric halide effect in the presence of each of these dyes was explored in the most competitive medium. Janus Green B provided the ability to test in an aqueous testing medium making it ideal for further investigation yielding the spectral data in the presence of chloride salt (Figure 14).

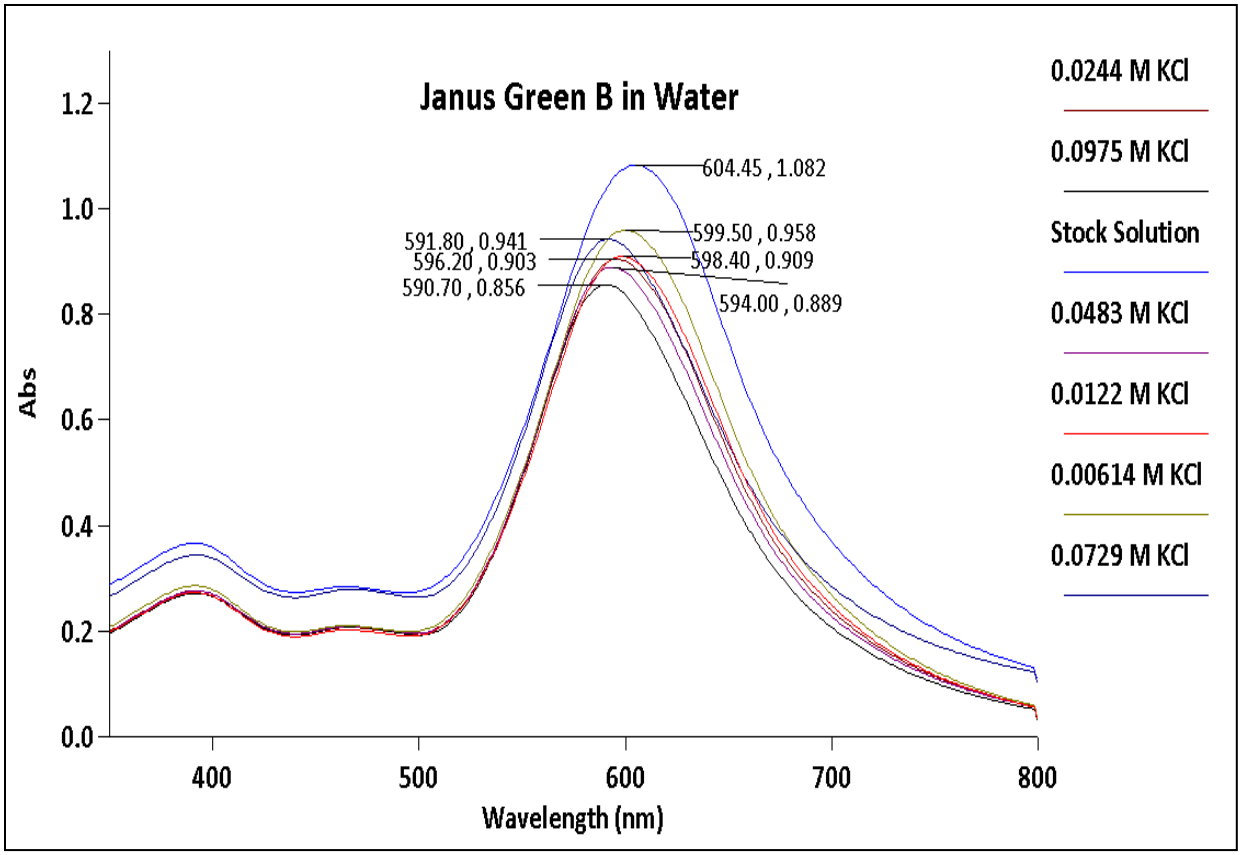


Figure 14. Janus Green B in water spectral response to bromide additions

The chloride response was relatively moderate with the most substantial peak maximum blue shift of about 14 nm. While this data was not entirely consistent with the previous qualitative measures, it does quantify the response with identified chloride additions. The discrepancies between the qualitative and quantitative tests could be attributed to the difference in cation (potassium versus sodium) and/or differences in the concentration of chloride introduced. Therefore, the only data comparison that can be made directly between the qualitative and quantitative was the presence of a peak shift with chloride additions. From these data a response curve was constructed to better visualize the solution's response to chloride additions (Figure 15).

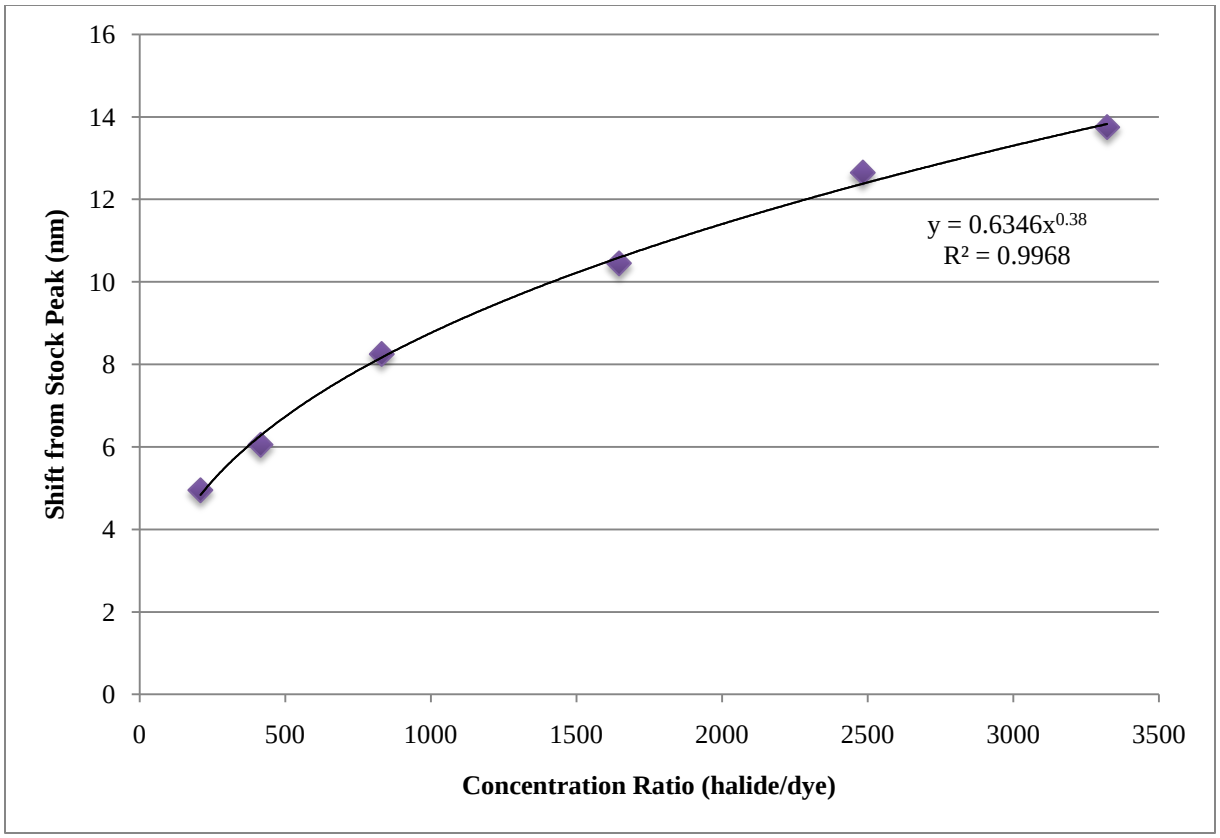


Figure 15. Janus Green B in water wavelength change response in the presence of chloride

The response curve was constructed from the peak maximum shift in the presence of chloride (relative to the stock Janus Green B peak) versus the ratio of the concentration of chloride added to the dye in solution. The dye-halide effect correlates to a non-linear response presenting some challenges for extracting mechanistic details. Since few concrete conclusions can be made as a result of the non-linear response combined with the present acid effect, structural implications were difficult to assess. This did not eliminate Janus Green B from contention for structural contributions though, because the survey goal was not to find a working indicator, rather molecules that might present advantageous synthetic motifs for halide detection.

Sudan Black B was also subjected to a more comprehensive examination of its colorimetric changes in the presence of bromide. Because of the aqueous solubility limitations, DMSO was again used to provide an analogous detection medium. However, any dye-halide attributed effect could not necessarily translate to aqueous detection as illustrated by the comparison of the results of qualitative CH_2Cl_2 and DMSO tests. Since the survey was centered on developing structural starting points for novel indicators, these conclusions were also largely unnecessary.

Sudan Black B's quantitative halide effect was probed in DMSO in the presence of acid bromide (Figure 16). As the concentration of the halide increased, the blue shift from the original peak maximum increased suggesting a more graded detection mechanism versus an "on-off" response mechanism.

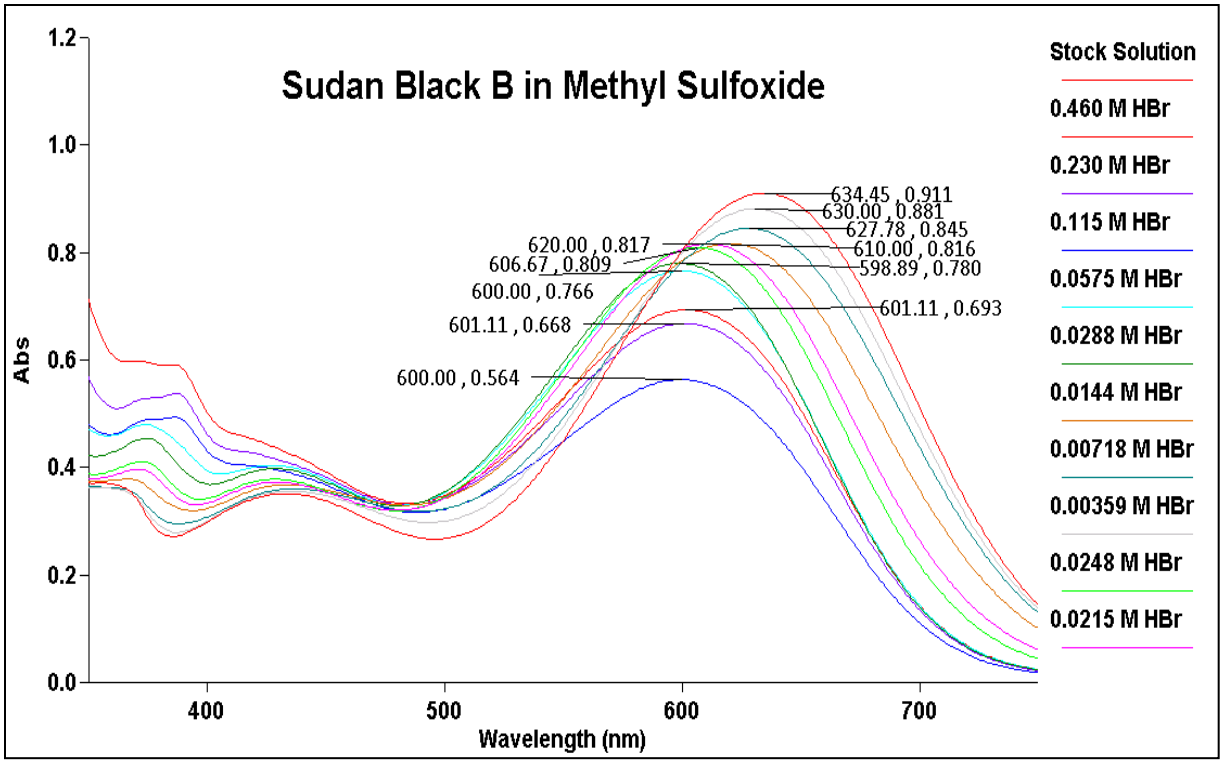


Figure 16. Sudan Black B in DMSO spectral response to bromide additions

The observed effect in the presence of bromide was anticipated, yet a notable spectral observation was the abrupt termination of any peak maxima shift around 600 nm despite increased bromide additions. This may have been a saturation effect, which is more apparent when plotted as a response curveFigure 17).

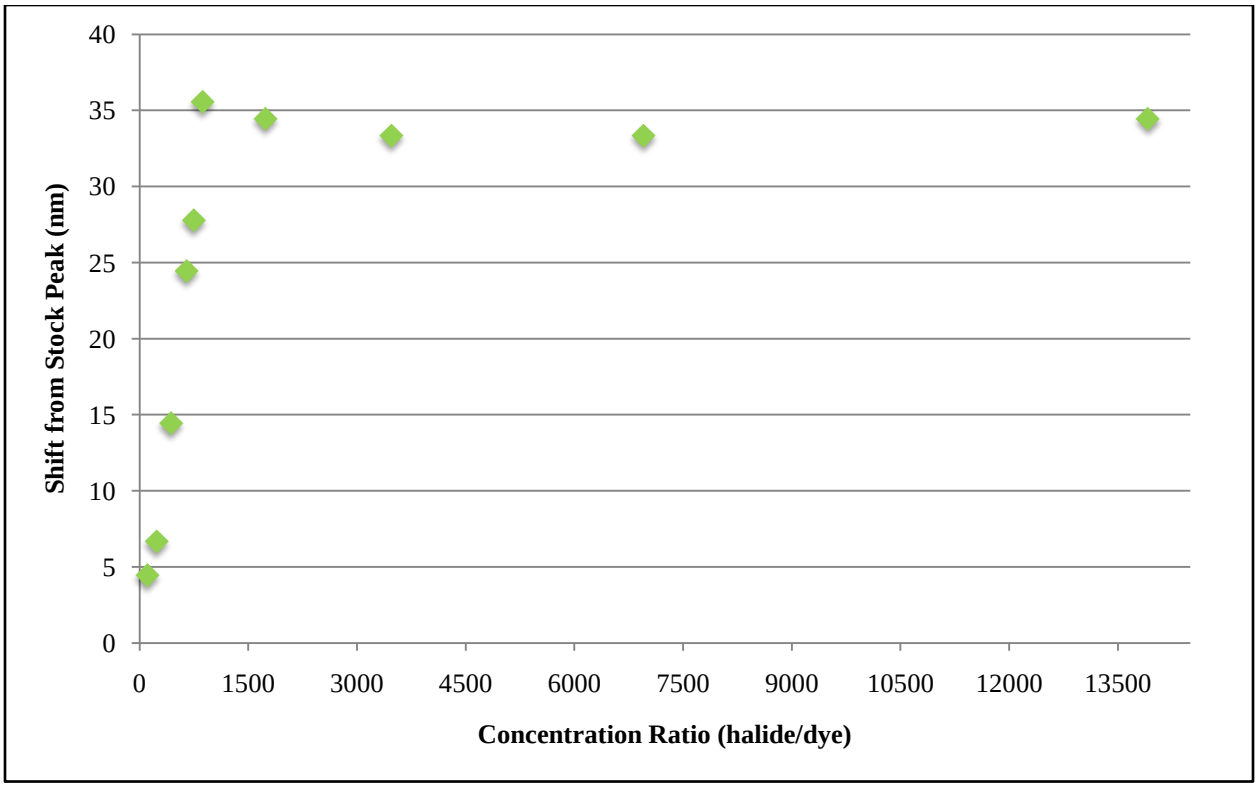


Figure 17. Sudan Black B wavelength change response in the presence of bromide

The response curve does not resemble the result of the Janus Green B quantitative tests, but this may be because as stated, the effect was likely saturated at high concentration ratios. To further examine the response, the region of points between no response and 900:1 halide-dye were expanded (Figure 18). This expansion yields not only a clear representation of the actual colorimetric response in the presence of halide, but a linear response to such introduction.

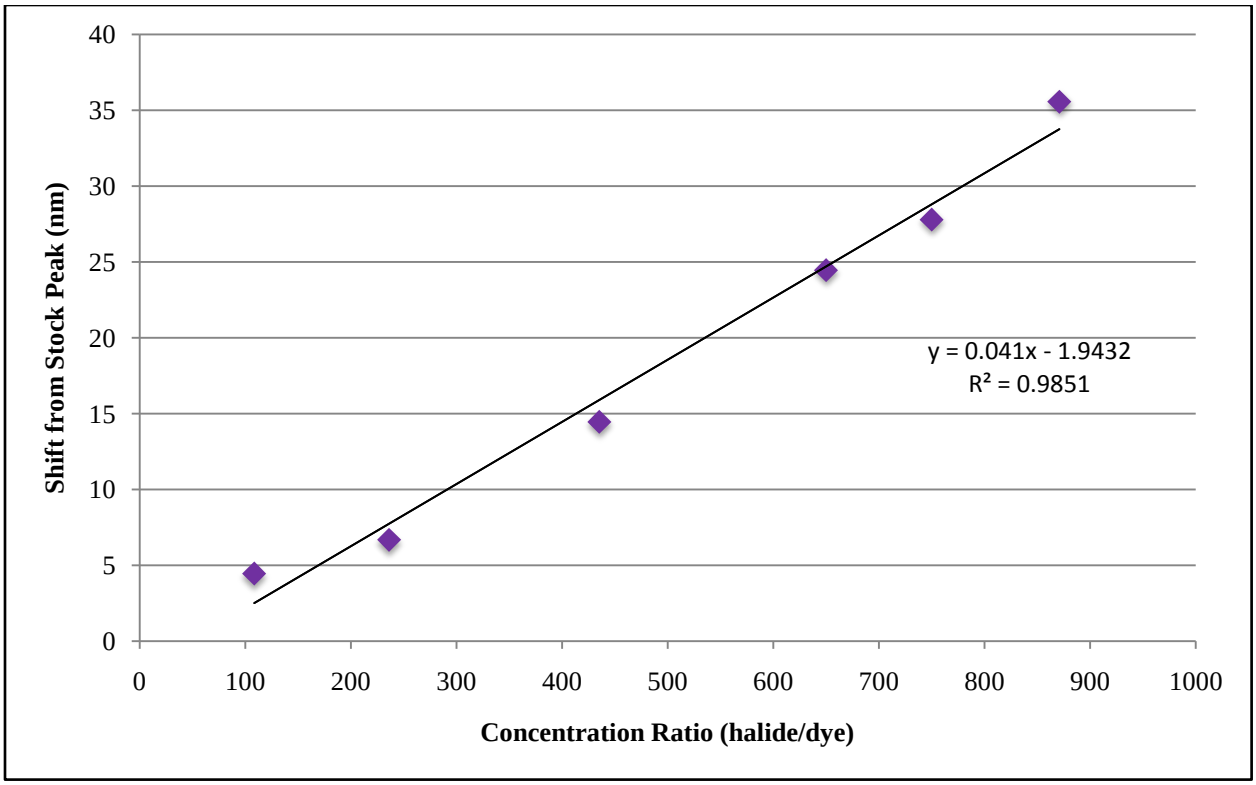


Figure 18. Excerpt of the Sudan Black B-bromide response curve

This linear response was more successful than expected and might suggest some mechanism of detection. However the lowest concentration ratio that elicited a response was quite high at about 100 times more halide than dye in solution. A number of explanations could be attributed to this response including a minor product contribution (commercial dyes are often impure) or some aggregate formation response in solution. These possibilities provide limitation, yet this particular response was more than successful for the goals of the commercial dye study. In DMSO, Sudan Black B provided a semi-selective response to bromide that was fairly distinguishable from acid, base, and altered ionic strengths. As such, this provided a synthetic basis for novel indicator development.

Analytical Commercial Dye Survey and Methodology Conclusions:

The analytical commercial dye survey not only served to provide structural motifs for further novel colorimetric indicator development, but also ushered in the necessity foraddressing a number of important testing variables. These variables include the consideration of proton concentration, activity, competitive anions, selectivity and sensitivity. Addressing these considerations throughout the survey proved important for understanding what structures would provide success and their limitations. These variables would continue to guide the determination of a successful colorimetric halide selective indicator for aqueous solution throughout the synthetic development process. Furthermore, focusing on this set of limitations as a standard methodology is a procedure the literature in this field needs. Full characterization within these parameters allows for proper indicator application and better mechanistic understanding.

Considering the results of this commercial dye survey, two azo-functionalized dyes were deemed successful for the purpose of novel synthetic indicator development. Sudan Black B and Janus Green B both provided qualitative and quantitative colorimetric shifts in the presence of bromide and chloride respectively. While each presented significant limitations, neither were characterized for the direct purpose of colorimetric halide detection allowing for some flexibility in how success was determined. Based on the survey spectral results, Sudan Black B was the more advantageous azo dye model for the development of a novel colorimetric halide indicator. Janus Green B provides a more accurate characterization of the halide effect as testing was completed in aqueous medium, however the structural complexity of the dye

coupled with difficult to characterize proton concentration effects limited its immediate utility. The determination of Sudan Black B as a more successful functional foundation was based on the results of the dye-halide studies in DMSO and the structural components of Sudan Black B. Though the point has been asserted that there is little guarantee a similar dye might perform in the same manner in an aqueous medium, further characterization of the structural components of Sudan Black B aims to achieve an environmental medium transcended effect with bromide. As the development of a colorimetric halide selective indicator for aqueous medium moves into the synthetic development realm, a more complete analysis of the functional components implicated in the literature for beneficial indicator properties necessitates completion. As such, this knowledge combined with the results of the commercial dye survey supports a successful avenue for the design of novel indicators with halide selective properties in aqueous medium.

Chapter 3

Structural Considerations and Implications for Aqueous Colorimetric Indicator Development:

It has been well established that there are a vast number of synthetic avenues in developing halide indicators that may be advantageous for aqueous detection. While that diversity has already been explored in an expansive literature review, the focus on colorimetric aqueous halide detection applies constraints to the possible structural candidates. Such limitations necessitate abandoning the consideration of molecules implicated in cation detection and large biological molecules while still considering non-colorimetric and non-aqueous features. This distinction is made because the field has diverged between anion and cation detection but has yet to make the same division with aqueous and colorimetric detection. Furthermore, synthetic goals of efficiency and low cost tend to eliminate the possibility of biologically based sensors due to their complexity.[51] Non-colorimetric detection includes the prominent field of fluorescent indicators, a relevant consideration particularly because of the possibility of dual properties that are often shared between fluorophores and chromophore.[3] Structurally, molecules such as these must be considered. Progress in the field has made separation of literature into either aqueous or non-aqueous detection nearly impossible.

The literature defines the molecular design of these indicators, especially fluorescence based indicators, in a very specific manner. Typically the structures follow the motif of a signaling unit attached to a binding unit with variation in the addition of a spacer unit between the two (Figure 19). The signaling unit often acts as a fluorophores or chromophore and the

binding unit undergoes some interaction with the halide in solution that causes the electronic environment to change which elicits an absorption or emission change. This is the fundamental view of a basic molecular indicator and has persisted.[1,2] While it has led to many successful indicators, the limitations of complexity and environment are still present and therefore leave room for improvement and modification.

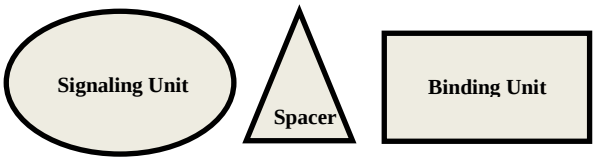


Figure 19. Signaling and binding unit model

Another structural issue is made apparent when delving into halide literature under the signaling and binding model. This model is seen across multiple anion detection methods, particularly in fluoride where often times the mechanism is or is similar to hydrogen bonding and in excess causes deprotonation.[14,30,20,52,53] The consequences of this are most certainly an absorption change if the sensor is colorimetric (emission if fluorescent), and it is relevant to consider the activity of the solution. The activity becomes a multifaceted problem that takes into account competitive media, pH changes, and interfering anions, and associated cations. If the mechanism mirrors hydrogen bonding, competitive media will most likely complicate the desired response. Furthermore, increasing the basicity of the solution will elicit the same response as fluoride, and this observation has aided in the development of a mechanisms of detection in these examples. Finally, ions like cyanide, that share similar properties to fluoride, can interfere easily making selective fluoride detection increasingly more difficult.[12,44,45,40] Because of these immense limitations in an aqueous environment, fluoride is often separated from the halide family in detection avenues and will be treated similarly in this structural study

and development. However, these examples highlight the complication in detection methods with interfering solution activity.

Similar to the issues presented with fluoride detection, many of the functional moieties of interest present an equal number of challenges to their positive attributes. For example, porphyrins offer colorimetric, selective halide detection capabilities but lack aqueous solubility.[25,26] Calixarenes present more flexibility in substitution abilities and therefore certainly improve the environmental limitations (DMSO solubility is reasonable). However, the complexity of these structures make it difficult to ascertain the mechanism of detection and can drastically increase synthetic complication.[27] The 1,8-naphthalimide structural unit presents more interesting indicator prospects particularly in the synthetic flexibility this piece provides as well as the availability for colorimetric indication. Highlighted in the review by Duke et al. (among other literature support) was the bis-functionalized approach.[1,28] This structural design provides increased complexity that may be directly related to an increased colorimetric effect. However, like many of the other molecules utilizing this structural component, the bis-substituted molecules compete with solubility and/or synthetic ease. Despite this, the bis-substituted 1,8-naphthalimides still support the utility of such a model as the basis for colorimetric halide detection design. Fluorescent studies have also introduced several functional groups for consideration including quinolinium, acridinium, and indole-based indicators (Figure 20) which have been a staple in the field.[46,50,54,47] These dyes do offer colorimetric possibilities but addressing water solubility and the complexity of the substitution would be absolutely necessary for aqueous halide detection.

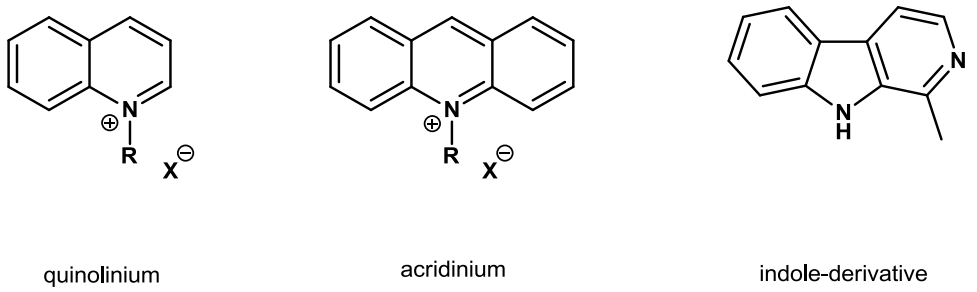


Figure 20. Fluorophore functional groups

With the filtered expectations of what would be structurally successful for aqueous colorimetric halide detection, several examples of pertinent functional motifs were explored for synthetic ease, expense, and environmental flexibility. Molecules such as porphyrins and calixarenes do provide the selectivity and sensitivity that is desired to some extent but are less valuable when considering aqueous detection. Simpler detection means were sought and this study began with the consideration of the broad reaching azo containing family of dyes. The azo functional group onsists of a nitrogen-nitrogen double bond bridging two (typically aromatic) substituents. Geometrically, the nitrogens and non-bonded electron pairs define a plane orthogonal to the nitrogen pi-bonding system (Figure 21). These molecules are typically synthesized through electrophilic aromatic substitution of aromatic rings with arenediazonium ions. Because a stable product of this reaction covalently bonds two substituted aromatic rings, the highly-conjugated products exhibit characteristic visible-light absorptions, making this family of dyes of particular interest as structural targets.[55]

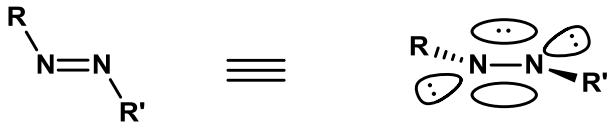


Figure 21. Azo Functional Group

Azo-functionalized dyes provide colorimetric properties yet have not been highly implicated in colorimetric anion detection thus far.[10,13] This may be due to their restricted use as a signaling unit and not essential in providing a specific interaction with the desired anion. By attempting to use these molecules as both the signaling unit and as a site of halide interaction, the previous molecular indicator model of separate signaling and binding units is challenged and a new synthetic avenue is opened. Implications of the azo functional group use are not all positive. For instance, whilethe current usage of azo dyes is expansive they also include a formidable deterrent in their representation as pH indicators. Methyl red is a classic example of an azo dye and is considered a commonly used pH indicator.[4] Methyl orange, shares a similar structure and therefore should also be used cautiously (Figure 22).[10]

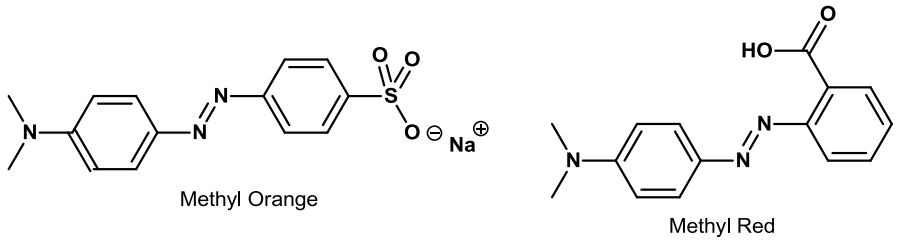


Figure 22. Azo Dyes as pH Indicators

Questioning the use of azo dyes becomes reasonable when considering possible competing pH effects, however when further examining these examples and others like them, two important features become apparent. Methyl orange and methyl red are both water soluble and synthetically simplistic. Designing an aqueous indicator should begin with addressing solubility to some extent because as previously discussed, solvent effects coupled with synthetic alterations may entirely alter the observed halide effect. Also, azo dyes are commonly synthesized via an azo coupling reaction from reactants that are commercially-available. As a result, the reaction can be achieved in a few short steps. Throughout the

analytical commercial dye study, the availability and diversity of azo dyes was apparent and advantageous. The properties of these dyes as colorimetric aqueous indicators overcome the possibility of pH and activity interference as limitations. Though one of the reigning models of anion detection would likely disregard this structural group as merely chromophores, blurring the line between the signaling and binding groups may provide a more simplistic and highly successful aqueous halide colorimetric indicator.

When addressing the structural features of a successful ionochromic aqueous indicator, several key features were identified as imperative. The previous analytical work recognized several possible functional moieties with Sudan Black B as the most important positive result. From this, the azo functional group became the central component in the construction of a novel halide indicator. To improve upon the limitations of the Sudan Black B, modifications were made to increase aqueous solubility, introduce structural simplicity, and amplify selectivity. Due to the lack of literature surrounding separation of product mixtures of or related to Sudan Black B, the focus of the synthetic work was initially in identification of a functional anion detection effect. However unconventional, the goal of this work is to characterize unique anion sensing effects, and though identification of a single molecular indicator is ideal, purification must be secondary for the sake of efficiency until such properties have been identified.

Synthetic Modification Survey Results and Discussion:

The analytical discussion of Sudan Black B proposed the utilization of its structural components for the design of an aqueous halide colorimetric indicator. This particular

molecule was implicated in detection of bromide and therefore will be the focus of the novel indicators developed. Sudan Black B, while sensitive to bromide, is not an ideal molecule to serve as an aqueous halide indicator as it is extremely insoluble in water. When considering what functional components are most pertinent for use in a novel indicator design, examination of what made Sudan Black B successful was necessary. Figure 23 illustrates the three members of the sudan dye family including Sudan Black B. This particular family is not structurally consistent nor did it provide spectrally consistent data (Table 1). Sudan Black B features an extended bis-azo relationship with no oxygen present and no proton sponge activity (defined by high basicity, proton absorbing qualities). These features may be correlated with the spectroscopic differences and therefore should be preserved in some sense when designing novel molecules.

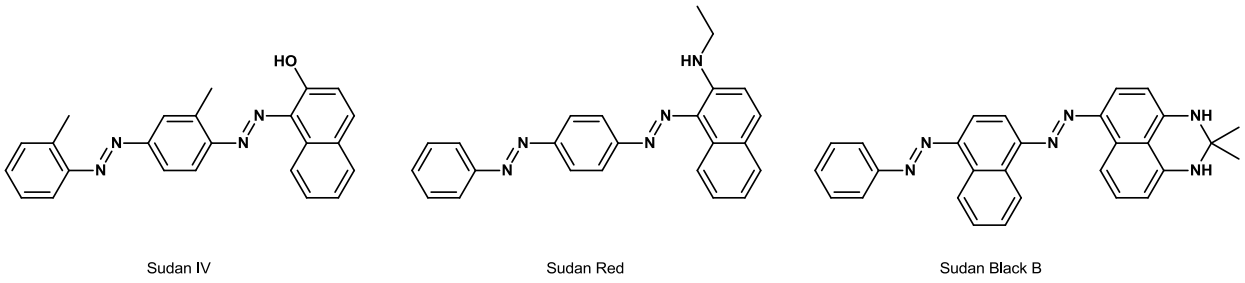


Figure 23. Structures from the sudan dye family

Consistent with the logic of the commercial dye analytical survey, a synthetic modification survey was designed to probe multiple structural modifications and their subsequent spectroscopic effects. This synthetic survey was conducted by organic chemistry students through the Research Experiences to Enhance Learning (REEL) project supported by National Science Foundation. The evolution of Sudan Black B from commercial dye to simplified

molecular indicator was centralized around the idea of exploiting a region of electron deficiency while preserving colorimetric characteristics (Figure 24). The "A" ring was deemed the electron deficient region of the molecule based on inferred electron withdrawing effects of the azo group. This electron-deficient region of the dye structure was hypothesized to provide a van der Waals surface attractive for an anionic interaction. The other substantial changes included removing the second azo group and its aromatic substituent for simplicity while removing the acetone aminal end group to leave a free naphthalene-1,8-diamine in order to increase water solubility. The overall effect of the novel synthetic design was improved aqueous solubility and synthetic ease through structural simplicity.

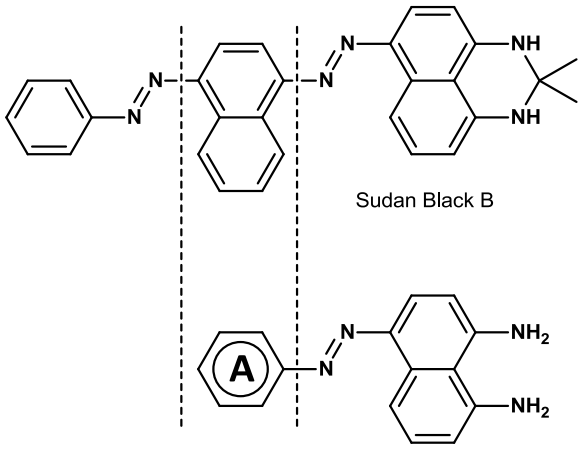
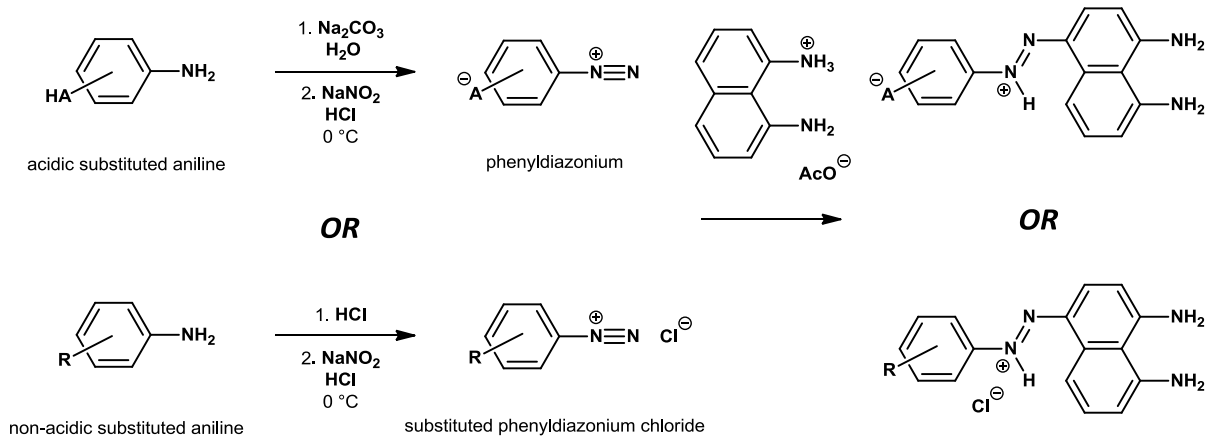


Figure 24. REEL Project structural skeleton

The synthetic scheme (Scheme 1) relies on the diazotization of substituted anilines (A-rings) followed by coupling with naphthalene-1,8-diamine.[55] This particular reaction was adapted from the well-documented synthesis of methyl orange, a common representative of the azo coupling synthesis. This two-step, one pot synthesis lent itself to the survey design particularly

for REEL project. Several commercially available substituted aniline reactants were chosen to exploit differences in substitution positions and functional properties. Each student focused on a particular substitution with the end goal of identifying an effect through qualitative spectroscopic examination in the presence of halides independent of pH. Purification was attempted through recrystallization, but the goal of this phase was limiting functional modification possibilities by identifying a colorimetric effect in the presence of a halide in solution and disregarding that with no consistent similar response.



Scheme 1. REEL Project Synthesis

The synthetic survey provided several functional modification options for further exploration, and of these modifications the nitroaniline, aminobenzenesulfonic acid, and aminobenzoic acid were deemed worthy of further investigation due to their initial promising REEL project data (Figure 25).

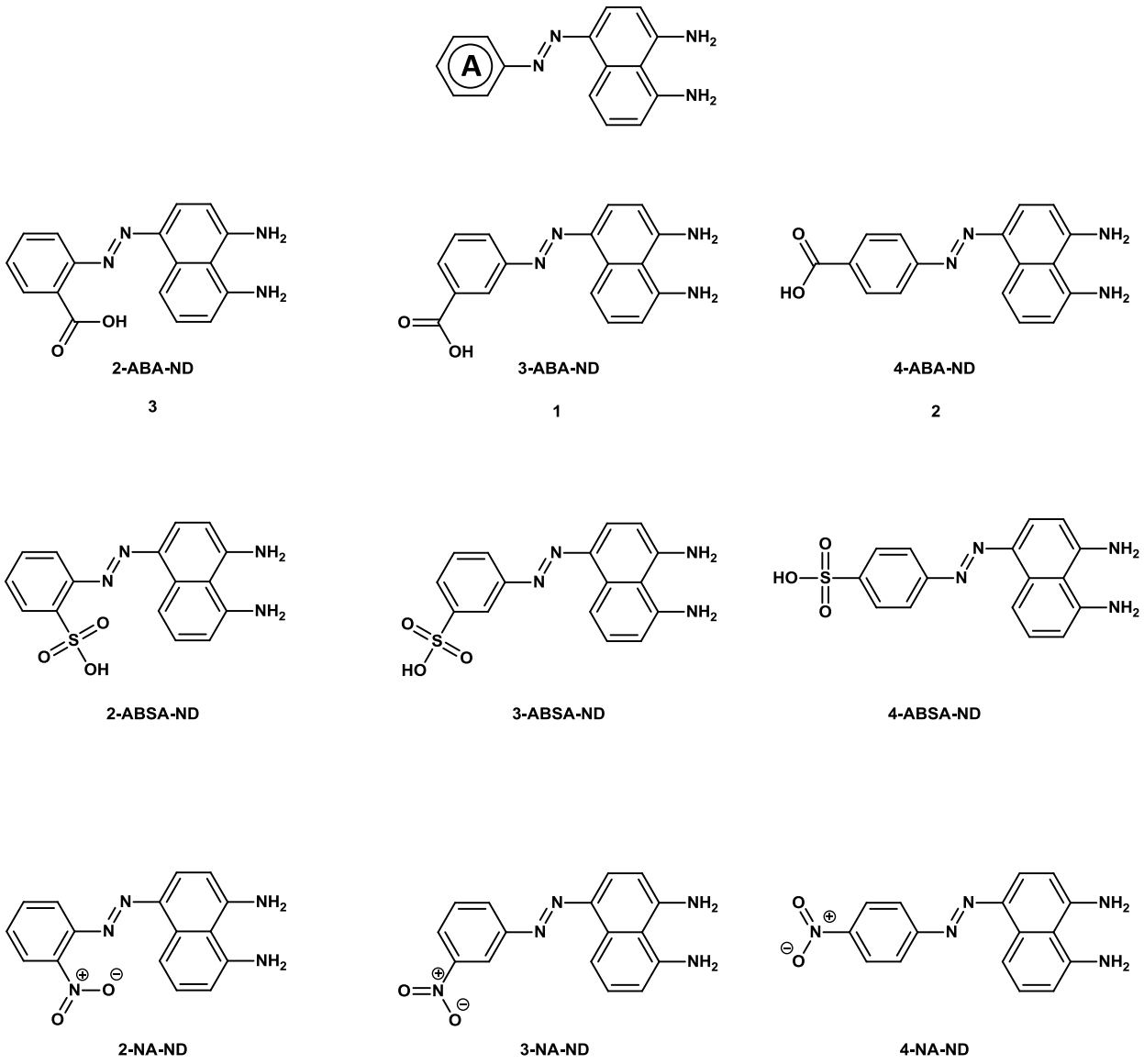


Figure 25. REEL Project Synthetic Targets

In particular, the meta-aminobenzoic acid-substituted product **(1)** provided a spectroscopic response to bromide in solution consistent with the original Sudan Black B data and consistent across several student groups (Table 2). Asides from addressing the most important goal of eliciting a colorimetric effect in the presence of a halide, this product had also increased aqueous solubility and synthetic ease from the original Sudan Black B motif.

Table 2. REEL Project Product 1 Results

Stock Solution in MSA λmax (nm)	Stock, MSA, NaBr λmax (nm)	Peak Maxima Change Δλmax (nm)
539	500	-51
541	504	-44
542	505	-36
548	505	-35
553	514	-33
534	501	-32
547	526	-27
533	527	-8
Average		
542	510	-33
Standard Deviation		
7	11	13

With this initial result, it became necessary to repeat the study in a more controlled manner utilizing the ortho-, para-, and meta-aminobenzoic acid substitutions to explore this effect and compare the specific modification position. Therefore, each aminobenzoic acid substituted product was synthesized, and as expected, the crude products proved difficult to purify via recrystallization though several attempts were made with variable success. Since a colorimetric effect in the presence of a halide was sought after more so than any other factor, the two products (crude and recrystallized) as well as the starting material were compared spectroscopically to discern noticeable differences (Figure 26). The resulting spectral comparison indicate no differences between the crude and recrystallized products, suggesting that halides studies using this recrystallized product would be appropriate.

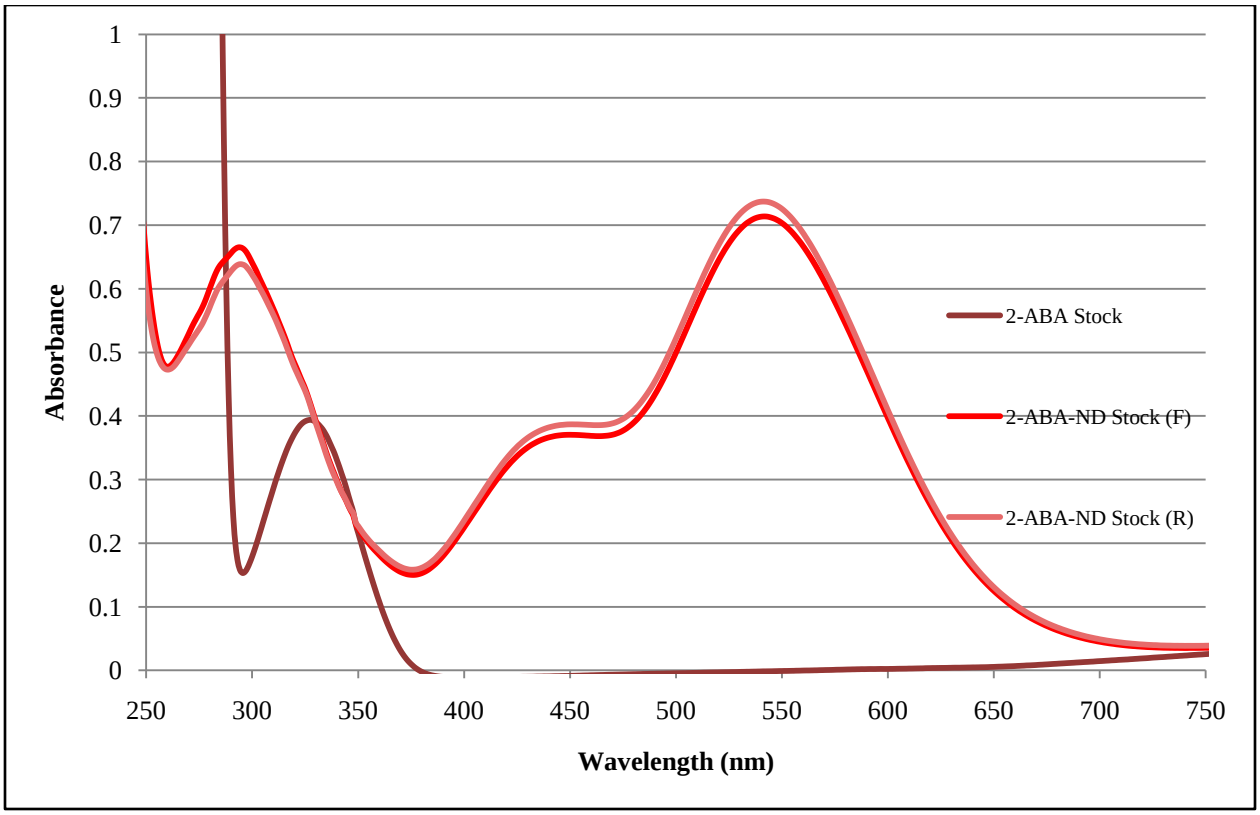


Figure 26. Recrystallized product 3 (2-ABA-ND) comparison to reactants and crude product

The qualitative dye halide tests were completed in a 0.1 M acidic aqueous environment to promote dye solubility. Halide introduction was completed with both acidic bromide and potassium bromide (as compared to sodium bromide in REEL project) (Figure 27). Comparison of the data could be made with the spectral data yielding information about important variables including activity (explored via different cations tested and acid additions).

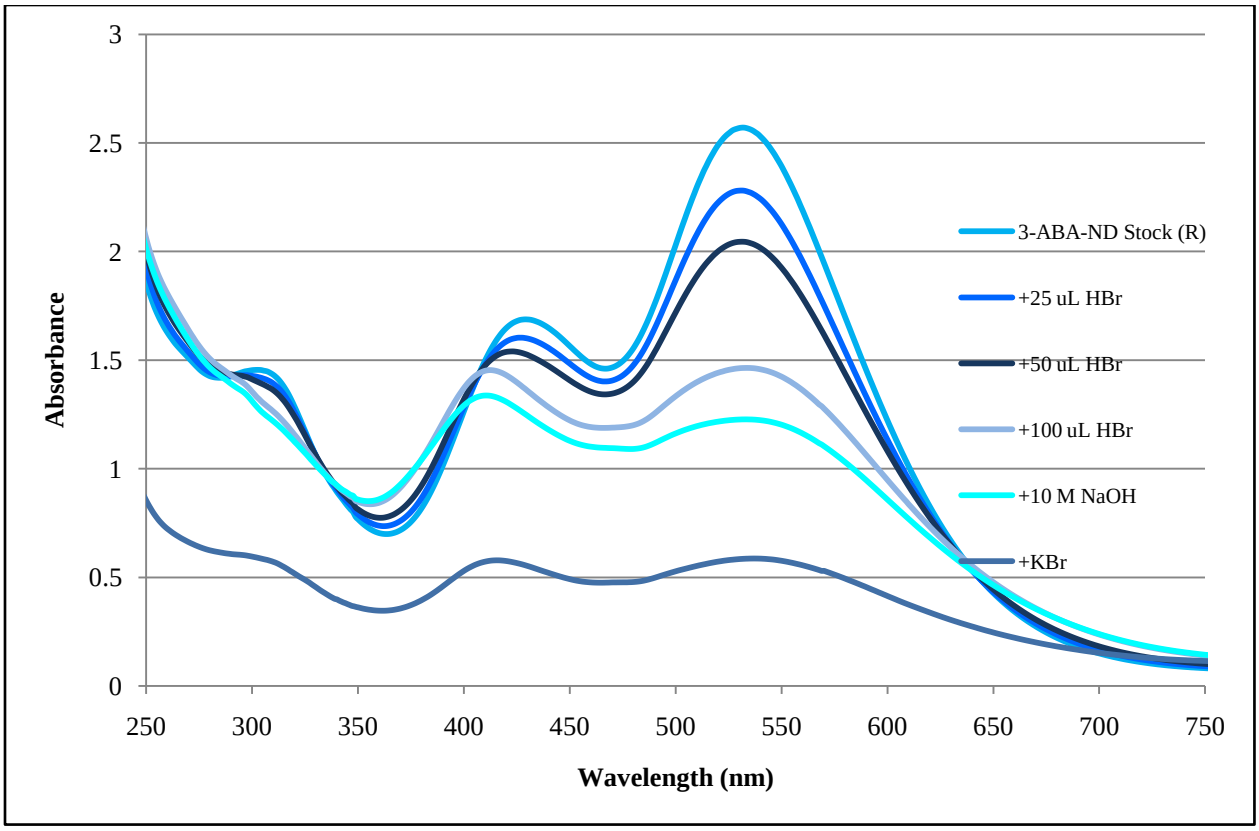


Figure 27. Product 1 (3-ABA-ND) Ion Tests in Aqueous Acidic Solution

The resulting colorimetric effect between compound **1** in solution and bromide was realized in the secondary peak at 428 nm blue shifting 20 nm. The magnitude of this shift was substantial enough to translate into a visual color difference and therefore was considered a viable colorimetric effect. This result was also similar to the effect seen with bromide and Sudan Black B in DMSO which both elicit a colorimetric blue shift of the peak maximum relative to the stock dye spectrum (Figure 28). However, these observations are not consistent with the data obtained by the Chemistry 255 students (Table 2).

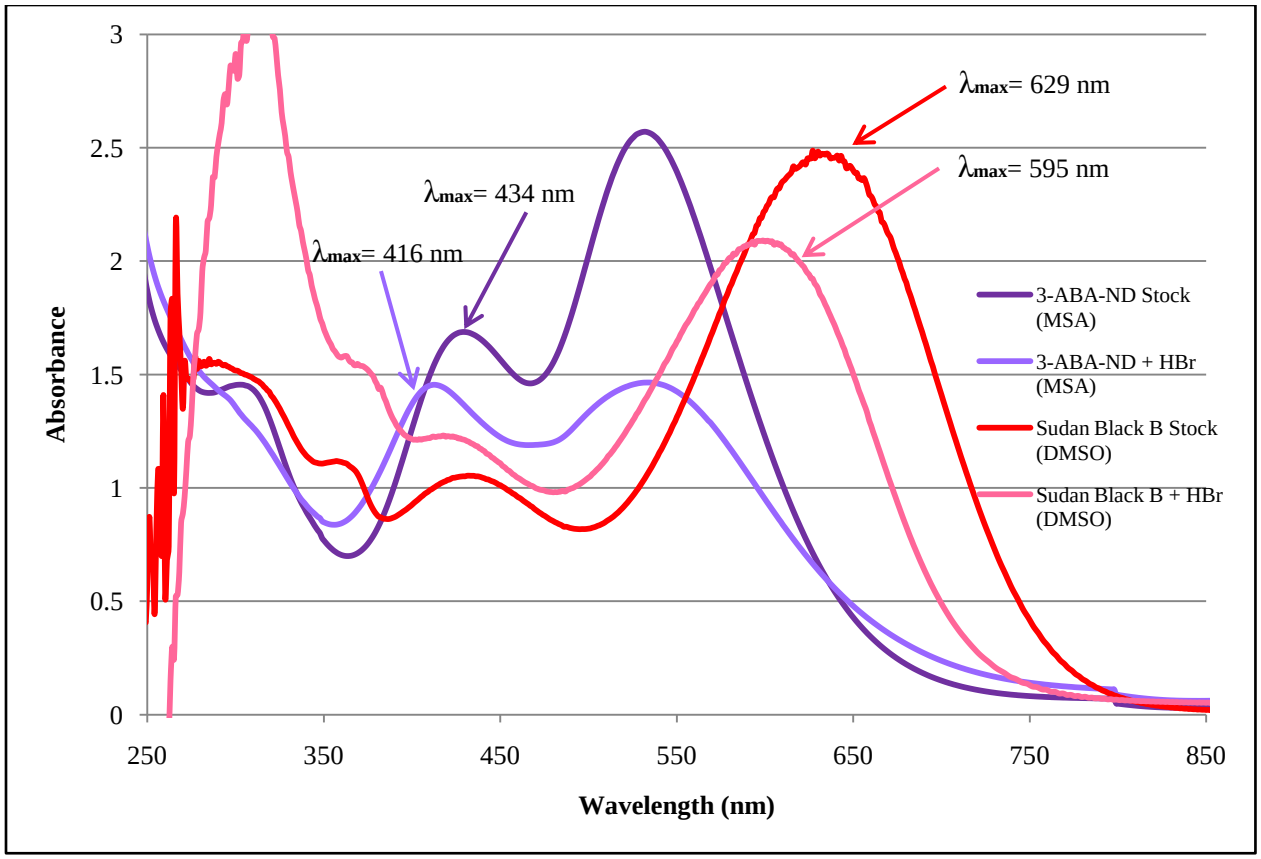


Figure 28. Sudan Black B Product 1 (3-ABA-ND) comparison with bromide response

Product 1's response with additions of bromide was compared to the spectra of additions of chloride in order to consider competing anion resulting in no perceptible wavelength shift. A final and imperative variable, proton concentration, was considered with competing additions of base eliciting little response until the solution pH reached basic. Ultimately large basic additions to the solution lead to large spectroscopic interferences due to dye solubility issues. This was expected considering the literature implications made about the importance of non-covalent hydrogen interactions in detecting halides coupled with the experimentally determined importance of a slightly acidic solution for optimal dye solubility. However, the importance of changes in proton concentration could not be ignored; this dye

and those similar were limited to the acidic and neutral pH. Molecules **2** and **3** showed little if any response with similar bromide additions and therefore were not further characterized.

At this point, the first synthetic goal of identifying a colorimetric halide effect had been met. While proton concentration remained an issue, it had been characterized qualitatively and the limitation of high pH effectiveness was identified. Purification had now taken precedence in order to further identify the single product responsible for the colorimetric response in the presence of halides and implicate the specific mechanism of detection. Complicated NMR and mass spectrometry spectra prompted discussion of other minor products that may have contributed to the solution. Literature review revealed not only the possibility of the presence of another product contribution but also that the synthesis procedure implemented in the synthesis of these compounds may favor multiple products. The following graphic (Figure 29) is an excerpt from literature demonstrating the probable presence of a bis functionalized molecule formed from an azo coupling reaction.[56]

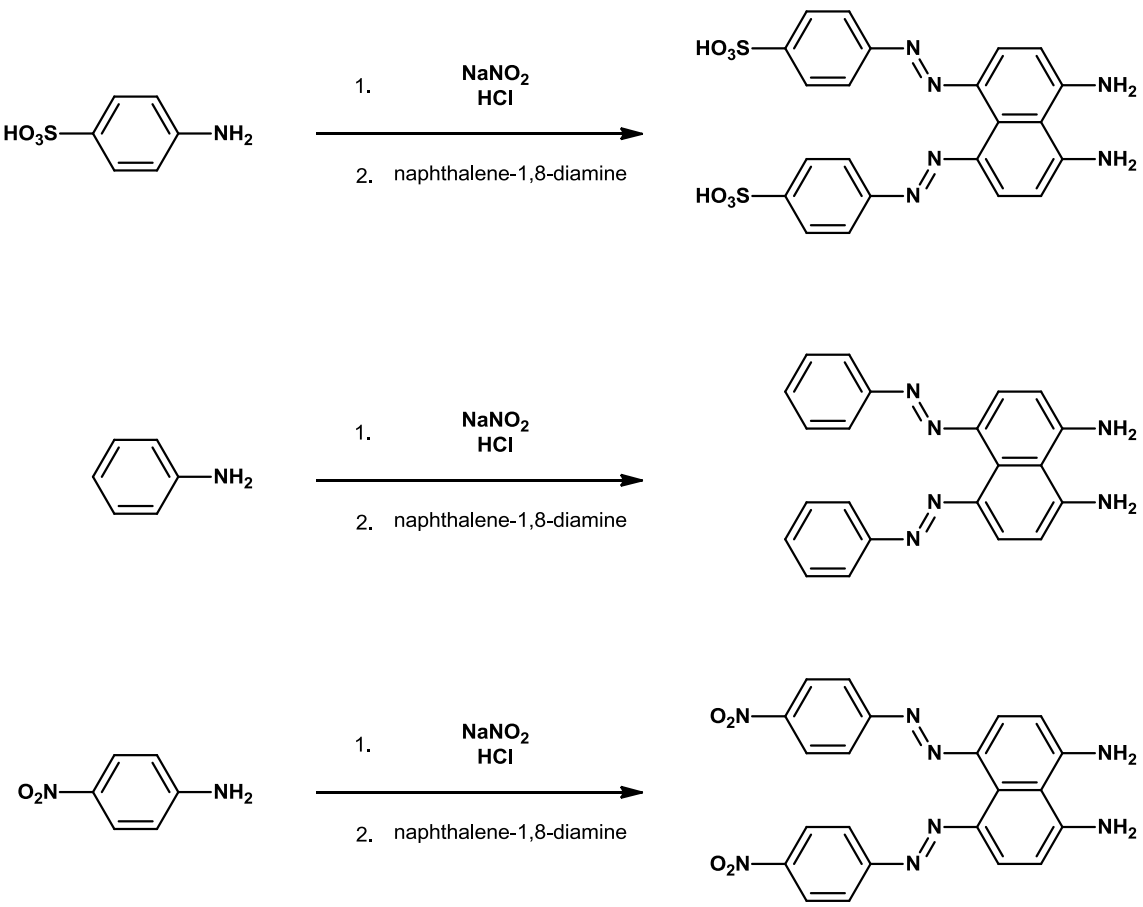
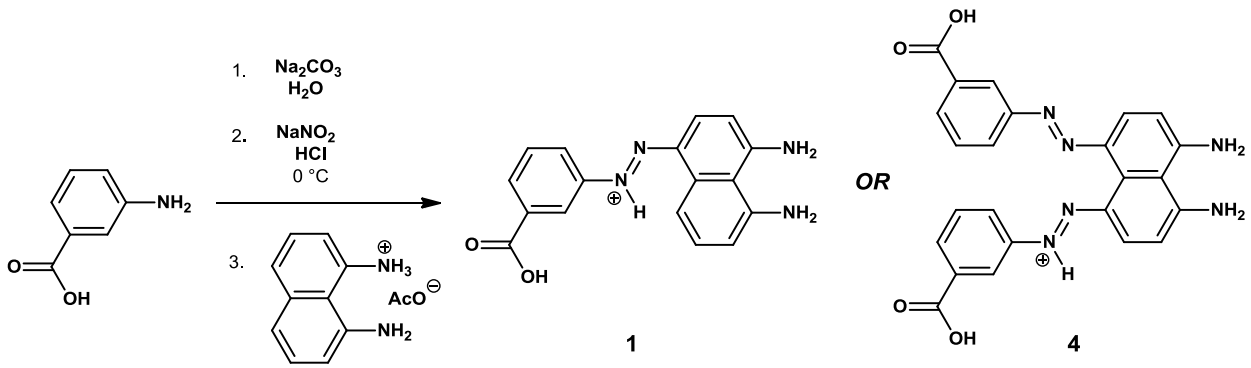


Figure 29. Synthetic routes supporting bis substituted product formation

The presence and possible colorimetric effect contribution of a bis functionalized minor product was supported in the colorimetric response of bromide additions of Sudan Black B. The concentration ratio of halide to dye was inordinately high to elicit a spectral response. Because of this, it was likely that this minor product could have been contributing, supporting the increased bromide presence (over 100 fold) required for a colorimetric response. These observations coupled with bis molecules implicated in synthesis and detection supports the need for further investigation and purification (Scheme 2).



Scheme 2. Product 1 (3-ABA-ND) Synthesis with Major and Minor Products

Initial Synthetic Survey Conclusions:

From the results of the initial analytical survey, Sudan Black B led to the development of a synthetic survey centered on the ease of the azo coupling synthesis and the goal of identifying a colorimetric effect in the presence of halides. This approach allowed for the vast REEL project synthetic modification survey to produce several avenues of success quickly, particularly with the aminobenzoic acid, aminosulfonic acid, and nitroamine modifications. Further synthetic development of the aminobenzoic acid products yielded a promising yet complex product composed of possibly contributing minor products. In an attempt to fully characterize the product of the aminobenzoic acid substituted reaction, a return to the literature was necessary and ultimately supported the presence of a doubly substituted product. This bis product model had been implicated in literature as both a likely minor product with similarities to advantageous indicator motifs. Synthetic modifications were necessary to probe the implications of the bis product's presence in the product solution. The bis product became a central focus of synthesis and fueled the increasingly paramount need for purified products.

Solely addressing purification, many of the substitution candidates from the synthetic survey were quite water soluble, making it difficult to isolate products. Purification and characterization as a primary synthetic goal had always been secondary to the effect but now was deemed necessary particularly for the aminobenzoic acid substituted product. Through these measures better understanding of the colorimetric effect could be achieved and more efficient purification methods for other novel azo indicator molecules could be developed.

Further Novel Dye Characterization Results and Discussion:

The doubly substituted 3-aminobenzoic acid product **4** demanded attention due to its literature support as an indicator motif and synthetic product. The presence of **4** was confirmed by LC-TOF results (M+H=307.12) and due to the previous indicator model implications, an alternative synthetic avenue was explored to further investigate the bis molecule's role in detection. The altered synthesis favored compound **4** to some extent while still producing a significant amount of compound **1**. This change in product population was enough to induce spectral changes and a similar response in the presence of bromide (Figure 30). The maximum peak shift was of a similar magnitude at 32 nm and further supported the necessary presence of the bis product for the colorimetric shift in the presence of bromide.

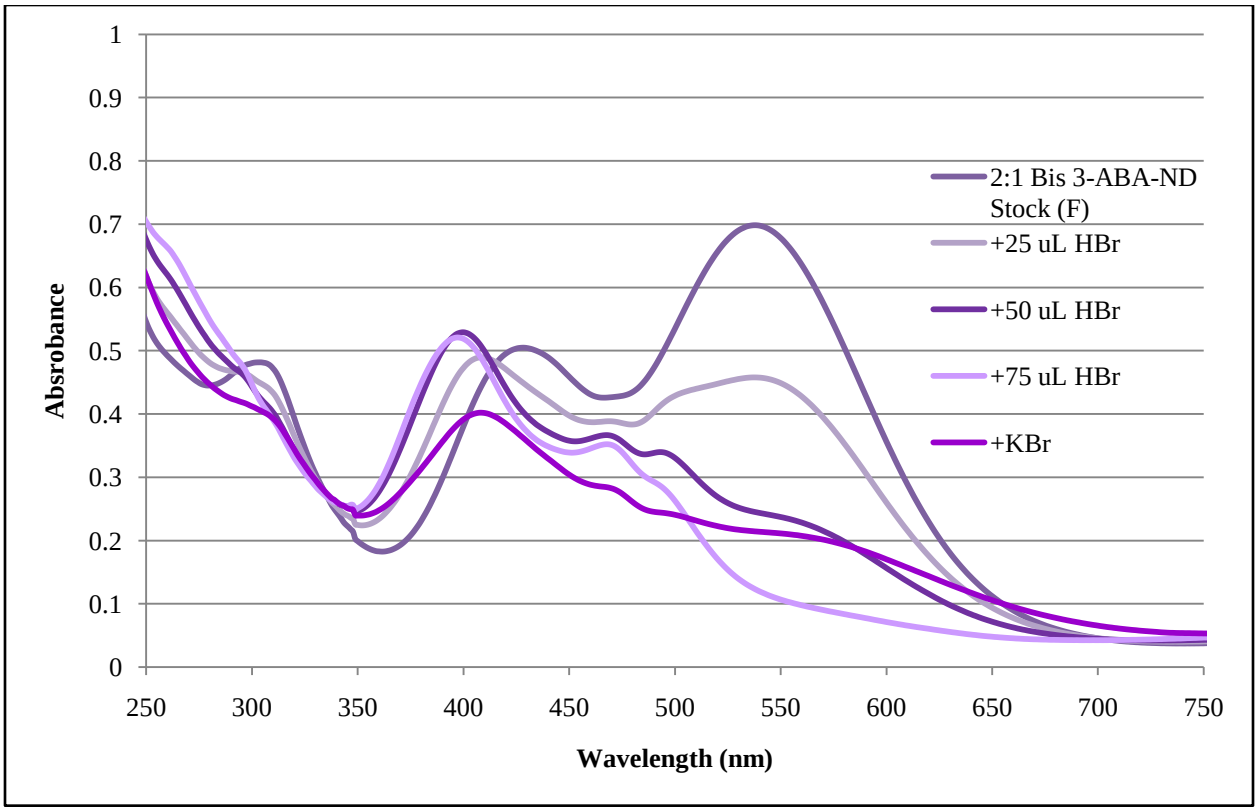


Figure 30. Bis substituted synthesis product 4 halide tests in aqueous acidic solution

Also, because of the shift in the population of the reaction product, the effect of altering the proton concentration was again considered (Figure 31).

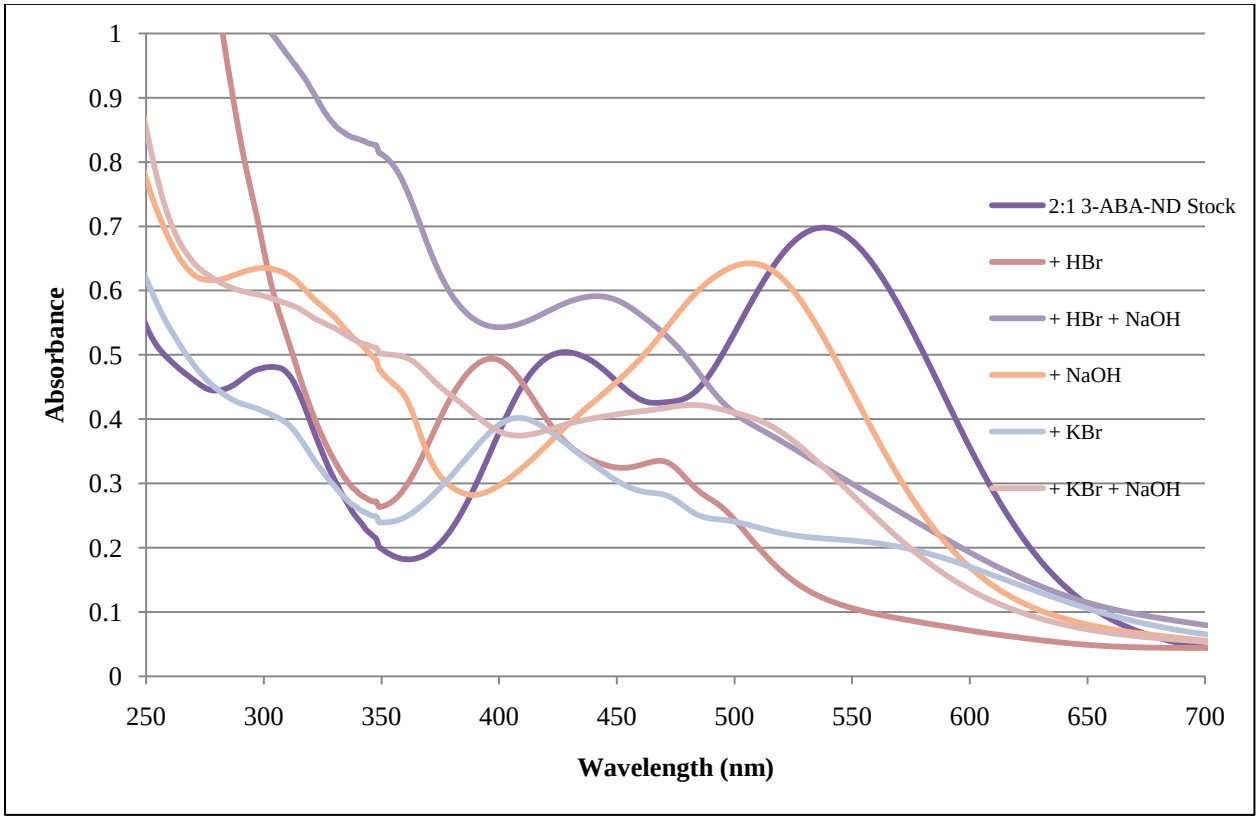


Figure 31. Bis substituted synthesis product 4 in the presence of acid, halide salt, and base

The spectral changes particularly in probing the effect of multiple additions of base (ultimately reaching a basic solution pH) illustrated a similar result seen with the predominately **1** product in basic conditions. As such, high pH continued to be a limitation of the aminobenzoic acid substituted products as colorimetric halide indicators in aqueous solution.

While this was not a definitive study due to product impurity and qualitative procedure, it does further support the hypothesis that product **4** contributed to the original halide effect identified in the REEL project as well as in literature with analogous indicators. The scale of the

interaction or difference in iteration was not well characterized, but is a target for continued experimental study. A more complex solution interaction such as an aggregate was also not ruled out. The source of the complexity may also be due to the presence of multiple isomers seen in the number of smaller peaks in the spectra of the more **4** product favored solutions. These isomers' presence were more concretely identified with chromatography and entirely relevant to more accurately characterize the solution.

The aminobenzoic substituted product solution population was initially probed to shed light on the photoisomerization properties and how they might relate to the interaction with bromide. The spectra below (Figure 32) are a visualization of isolating the product solution in the dark for three days then subjecting it to subsequent bromide additions.

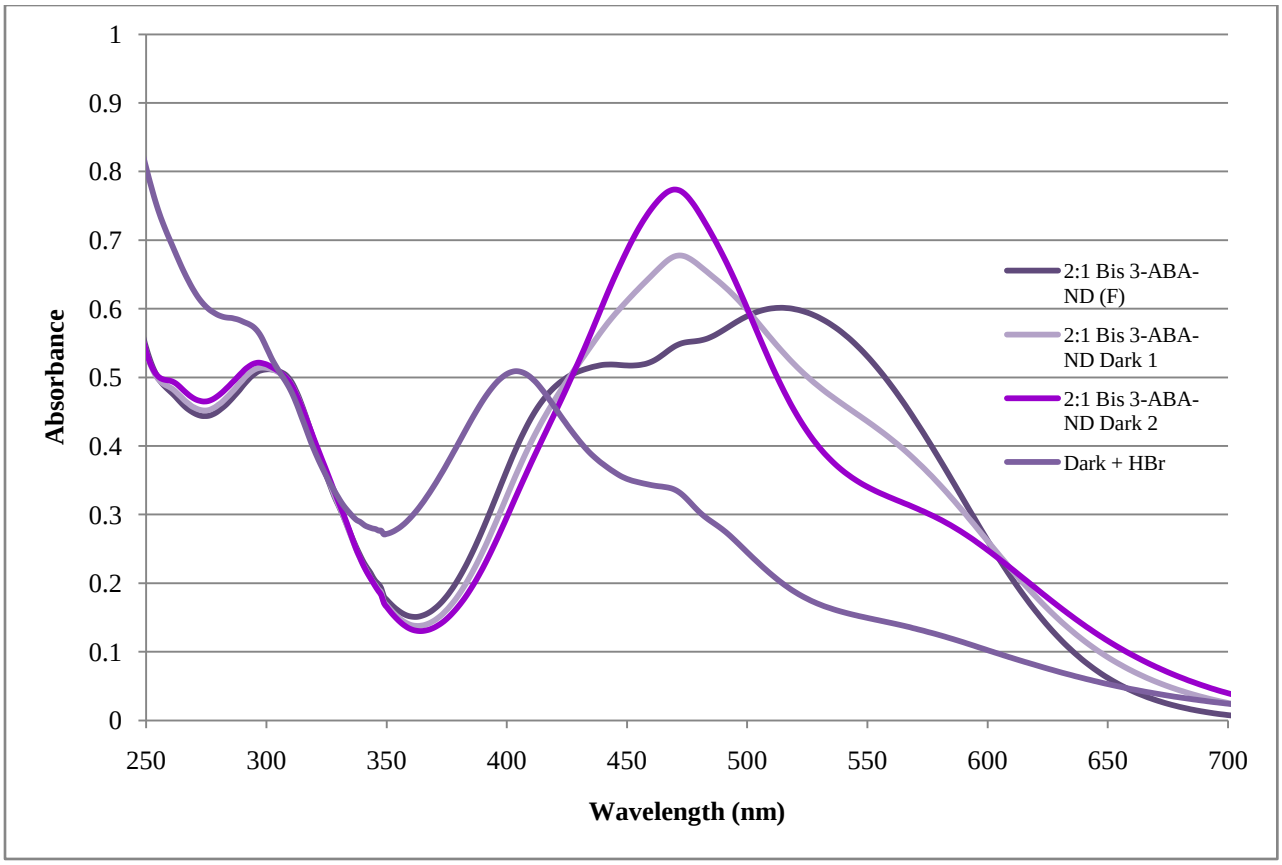


Figure 32. Dark Incubation of the bis substituted synthesis product 4

Interestingly, the dark incubated bromide-dye spectrum was similar to the spectra acquired with the dye-bromide solution exposed light. This evidence suggested cis-trans isomerization equilibrium may be shifted in the presence of certain anions.

With contributing isomers and multiple minor products, purification became daunting and required the power and precision of preparatory HPLC for product separation. The resulting product isolated was the major reaction product **1**. This particular purification technique yielded a small amount of pure product of about 10 mg total leaving it within the

bounds of limited characterization and quantitative testing. Due to these limitations, LC-TOF was utilized for product identification and purity assessment.

The subsequent spectroscopic tests of the pure compound **1** were qualitative yet nonetheless exceptionally enlightening. The spectra show the novel dye in solution and subsequent additions of bromide, chloride, acid, and base (Figure 33).

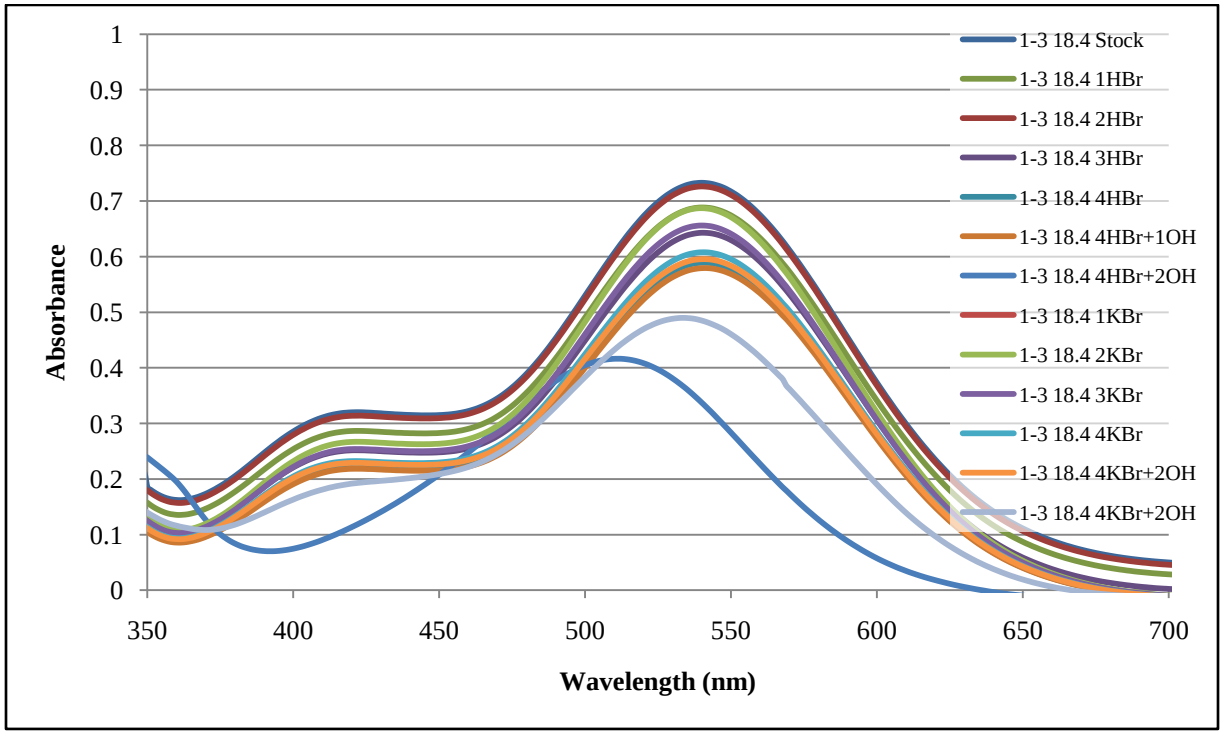


Figure 33. Purified mono substituted product 4in acidic aqueous solution

The characteristic colorimetric shift seen in the original product solution was curiously absent, in fact, there was little differentiation between bromide and chloride additions and the most dramatic shift was seen only with increased pH of the solution. Product **1** showed no selectivity, sensitivity, and was pH sensitive—none of which was deemed advantageous or consistent with the recrystallized product's spectral results.

Another peculiarity of this product was realized via the analysis of the LC-TOF data with the presence of three peaks being correlated with the expected product's mass to charge ratio at three different retention times (2.61 min, 4.01 min, 7.96 min). It was contributed to the presence of isomers, but the difference in retention times were larger than expected and therefore supported the likelihood that these isomers were most likely regioisomers Table 3 below shows the relative peak areas of the pure product **1** separated by preparatory HPLC, analyzed and quantified by UHPLC (detected by UV-Vis at 430 nm) and verified by TOF-MS at M+H 307.12 m/z. The presence of these stereoisomers support the need for alterations in the original synthesis as it produced too complex a mixture to gain a high yield of separable products.

Table 3. LC-TOF Analysis of Product 1 Isomers

Fraction Collected on Prep HPLC (retention time)	**Retention time of UHPLC-TOF**		
	2.61 min	4.01 min	7.96 min
18.5 min	100%	0%	0%
19.7 min	0%	94%	6%
23 min	0%	77%	33%
29 min	0%	0%	100%

The spectroscopic results of the purified mono substituted product **(1)** provided more support for the importance of the bis-functionalized product **(4)** as the primary detection molecule for bromide. While it has not been fully implicated due to low-purified product yield, the data supported the fact that major product of the reaction *alone* did not elicit the original identified colorimetric effect in the presence of bromide. This conclusion demanded improved

synthetic conditions for increased product yield and bis-functionalized product formation. Simultaneously, purification must also be addressed and aimed to be focused on reaction conditions that favor easily isolated products through synthesis modifications.

Synthetic modifications required a retreat to literature for alternative reaction conditions. Several factors contributed to the complications of the reaction and the need to address what was hindering reactivity lay at the core of the main issue. A component of the molecule initially addressed and soon secondary to the A-ring substitutions (Figure 24)was the naphthalene-1,8-diamine functional group altered from the original acetone aminal end group. This change seemed innocuous allowing for improved solubility and decreased synthetic complexity, yet may have affected the reactivity of the naphthalene ring in the azo-coupling reaction. The synthetic conditions to this point had been aqueous and as such protonated species were favored. Particularly when comparing the varied end groups explored through REEL projects, (Figure 34) protonation of these species may result in a decrease in the azo coupling reaction rate.

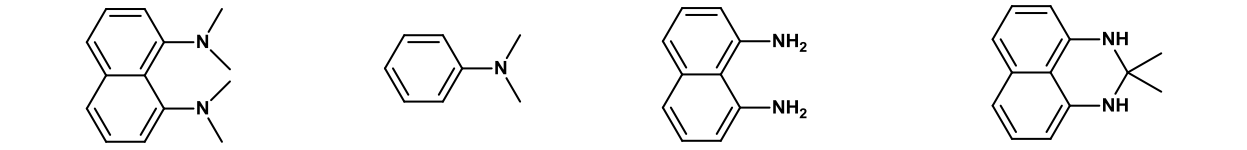
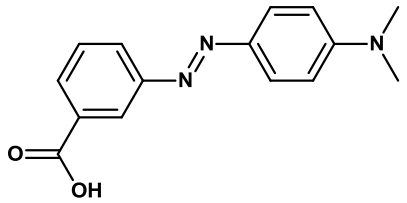


Figure 34. End group substitutions for novel dye development

The original end group of Sudan Black B (acetone aminal), was hypothesized to be not nearly as affected by the presence of acid and water during the reaction as the end groups naphthalene-1,8-diamine and tetramethylnaphthalene-1,8-diamine (which has proton sponge

properties). These species became extremely difficult to deprotonate, making them difficult to react particularly when considering how protonating the molecule affects the electron conjugation and therefore the reactivity of the end group. The realization occurred when more recent iteration of the REEL project resulted in mostly unreacted tetramethylnaphthalene-1,8-diamine. These issues made reconsidering what end group was best employed as well as prioritizing the reduction of the aqueous synthetic environment.

Several literature citations have ushered in a number of novel approaches particularly in reaction conditions consisting of nearly an entirely organic medium. [57] The azo coupling reaction in majority non aqueous medium had relative success in synthesizing the molecule **5** with increased yield of crystalline product.



5

Figure 35. 3-aminobenzoic acid dimethylaniline substituted product (5)

This improvement has allowed for the possibility of testing more conventional and less demanding purification techniques while under continual investigation.

Final Synthetic Modification and Novel Dye Characterization Conclusions:

By altering the reaction scheme, issues of purity and product yield have begun to be addressed. With the strong implications of the importance of the bis substituted product **(4)**, a reaction scheme must be developed to favor this formation in order to isolate a pure product

for spectroscopic tests. With the aid of the majority non-aqueous synthetic environment, this task as well as utilization of synthetic modifications such as the use of the tetramethylnaphthalene-1,8-diamine end group can be realized.

Throughout the synthetic development process, the most prominent goal remained the same—find a colorimetric effect independent of pH. This initial goal has been met through the identification of several promising substitutions associated with bromide detection. Further purification and varied synthetic modifications aim to target solution characterization in order to better understand the mechanism of the effect. Qualitative testing yielded results that ultimately limited the exploration of sensitivity. Once these synthetic obstacles have been addressed, subjecting each novel indicator to the analytical testing methodology developed for characterization is imperative. The resulting information coupled with molecular modeling techniques could provide support for the determination of a mechanism for this interaction between the dye and halide.

Experimental Procedures:

REEL project acid substituted aniline product synthesis:

The acidic substituted aniline and sodium carbonate were suspended in 4 mL of water and heated to near boiling to dissolve all solids. The solution was cooled to rt on ice and 0.15 g of sodium nitrite was added and stirred until dissolved. This solution was then added to 7 g of ice and 1 mL of concentrated hydrochloric acid. The phenyldiazonium precipitate forms after 1 minute and was added to a solution of 0.33 naphthalene-1,8-diamine in 0.5 mL glacial acetic acid. The reaction mixture was stirred for 15 min at rt then neutralized with sodium hydroxide.

The product was recrystallized in hot water and isolated via vacuum filtration rinsed with ice cold saturated sodium chloride.

REEL project Qualitative UV-Vis analysis:

Products were dissolved in water or 95% EtOH plus 0.01 M methanesulfonic acid and the concentration was adjusted to achieve an absorbance value between 1.2-1.8. To acidify the dye solution, 0.01 M methanesulfonic acid (MsOH) was added and all color changes noted. The spectrum of the solution was recorded using Ocean Optics USB-650-VIS/NIR utilizing a 650 element diode array detector and the λ_{max} and absorbance noted. The solution was then subjected to qualitative additions of 0.010 NaCl solution or 0.010 NaBr solution and the visible spectrum recorded after each addition. After the subsequent additions of a halide, activity and pH changes were tested through additions of 0.2 M NaOH while noting any visible color and/or spectral changes.

UV-Visible Instrumentation:

UV-Visible spectral analysis was completed with a Varian Cary 5000 UV-Vis-NIR spectrophotometer scanning 175 nm-800 nm at 600.00 nm/min.

UV-Vis Qualitative Analysis:

Solutions were prepared with 0.10 M methanesulfonic acid aqueous solution for absorbance values between 0.5-3 units. Additions of acidic halide (HBr, HCl) were made via micropipette and a subsequent spectrum recorded for each addition. Following the acid halide additions, NaOH was added incrementally until the solution was basic with a spectrum

recorded following each addition. After each individual halide test and subsequent pH test with base additions, the cuvette was clean and the procedure was repeated with each halide. Halide salt additions (KCl, KBr) were made to saturation and the resulting spectrum compared to the acidic halide additions.

LC-TOF Instrumentation

Structural analysis completed with an Agilent 1200 Series HPLC with a ZORBAX Eclipse Plus Narrow Bore Rapid Resolution, 1200 bar (2.1 x 150 mm, 1.8 um particle size) column and an Agilent 6224 TOF LC/MS with ESI.

Preparatory HPLC and LC-TOF Prep HPLC Sample Analysis:

The crude product was separated via preparatory HPLC with a reverse phase (C8) column. The mobile phase was comprised of a gradient of 10:90 CH_3CN:H_2O to 50:50 CH_3CN: H_2O over 30 min.

Analysis of the pure products was completed with LC-TOF (ESI). The mobile phase conditions was a gradient from 80:20 H_2O:CH_3CN (0.1% formic acid) to 20:80 H_2O:CH_3CN (0.1% formic acid) over 20 min.

LC-TOF Run Parameters:

Flow rate: 0.2 ml/min, Column temperature: 25°C, Drying gas: 10 L/min, Nebulizer: 40 psig, Sheath gas temperature: 325°C, Sheath gas flow: 12 L/min

ESI parameters: VCap: 2000 V, Nozzle voltage: 0 V

2-, 3-, 4-aminobenzoic acid substituted product (mono) synthesis:

Aminobenzoic acid and sodium carbonate were suspended in 4 mL of water and heated to near boiling to dissolve all solids. The solution was cooled to rt on ice and 0.15 g of sodium nitrite was added and stirred until dissolved. This solution was then added to 7 g of ice and 1 mL of concentrated hydrochloric acid. The phenyldiazonium precipitate forms after 1 minute and was added to a solution of 0.33 naphthalene-1,8-diamine in 0.5 mL glacial acetic acid. The reaction mixture was stirred for 45 min at rt then neutralized with sodium hydroxide. The product was recrystallized in hot water and isolated via vacuum filtration.

Modified 3-aminobenzoic acid substituted product (bis-favored) synthesis:

Aminobenzoic acid and sodium carbonate were suspended in excess water and heated to near boiling to dissolve all solids. The solution was cooled to rt on ice and 0.15 g of sodium nitrite was added and stirred until dissolved. This solution was then added to 7 g of ice and 6 mL of concentrated hydrochloric acid. The phenyldiazonium precipitate forms after 1 minute and a 0.33 naphthalene-1,8-diamine in 0.5 mL glacial acetic acid solution was added. The reaction mixture was stirred for 50 min at rt then neutralized with excess sodium hydroxide. The product was difficult to recrystallize therefore the product was instead isolated via vacuum filtration.

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